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Der Präsident des Europäischen Patentamts;  
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Prevention, treatment and diagnosis of diseases associated with Beta-amyloid  
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# **PREVENTION, TREATMENT AND DIAGNOSIS OF DISEASES ASSOCIATED WITH $\beta$ -AMYLOID FORMATION AND/OR AGGREGATION**

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## **FIELD OF THE INVENTION**

The present invention relates to the prevention, treatment and diagnosis of diseases associated with  $\beta$ -amyloid formation and/or aggregation. More particularly, the 10 present invention provides new  $\beta$ -amyloid (A $\beta$ ) and amyloid precursor protein (APP) peptides and new antibodies recognizing said A $\beta$  and APP peptides for use in the prevention, treatment and diagnosis of diseases associated with  $\beta$ -amyloid formation and/or aggregation.

15

## **BACKGROUND ART**

Amyloidosis refers to a pathological condition in a mammal characterized by the presence of amyloid fibers. Amyloid is a generic term referring to a group of diverse 20 but specific protein deposits. All amyloid deposits have common morphologic properties, stain with specific dyes (e.g. Congo red), and have a characteristic red-green birefringent appearance in polarized light after staining. Different amyloids are also characterized by the type of protein present in the deposit. For example, neurodegenerative diseases such as scrapie, bovine spongiform encephalitis, 25 Creutzfeldt-Jakob disease and the like are characterized by the appearance and accumulation of a protease-resistant form of prion protein (referred to as ASv or PrP-27) in the central nervous system. Similarly, Alzheimer's disease, another neurodegenerative disorder, is characterized by neuritic plaques and neurofibrillary tangles. In this case, the plaque and blood vessel amyloid is formed by the deposition 30 of fibrillar A $\beta$  amyloid protein.

Alzheimer's disease (AD) is the most common type of senile dementia and is believed to be responsible for 70% of all cases of dementia. The incidence of AD increases with age, affecting 1 out of 10 persons older than age 65 and nearly 1 out of 2 persons older than age 85. The disease is characterized by cognitive disorders associated with

5 a loss of memory and orientation, and deterioration of the intellectual capacity of the patients (Duyckaerts et al., 1999). In view of the large expenses for health care systems that must provide institutional and ancillary care for the AD patients, the impact of AD on society and on national economies is enormous.

- 10 Two major types of histological lesions are observed in AD brains, in association with neuronal loss (Felician and Sandson, 1999): (i) at the intracellular level, the neuronal cytoskeleton in AD patients is progressively disrupted and replaced by neurofibrillary tangles (NFTs) composed of paired helical filaments (PHF); (ii) at the extracellular level, amyloid plaques are formed by deposits of fibrillary  $\beta$ -amyloid ( $A\beta$ ).

- 15 The microtubule-associated protein tau is a major protein component of paired helical filaments (PHF) and neurofibrillar tangles (NFT), associated with Alzheimer's disease (Brion et al., 1985; Delacourte and Defossez, 1986; Grundke-Iqbali et al., 1986; Kosik et al., 1986; Wood et al., 1986; Kondo et al., 1988). Ten stages of tau pathology (S0-  
20 S10) were defined, according to ten brain areas that are successively affected (Delacourte et al., 1999).

$A\beta$  is a major component of the senile plaques.  $A\beta$  is a small peptide found mainly in two sizes, 40 ( $A\beta_{40}$ ) and 42 ( $A\beta_{42}$ ) amino acids, and in minor amounts in other sizes.

- 25  $A\beta$  is known to be metabolised from the proteolytic cleavage of APP (Saido, 2000), a large transmembrane protein with known, although not completely clear, neurotrophic functions (Seo et al., 2001). APP can be cleaved via two main routes, a major non-amyloidogenic route and a minor second, amyloidogenic route that yields  $A\beta$  as ultimate product.

- 30 The main pathway for catabolism of APP is through cleavage by  $\alpha$ -secretase at a single site in APP near the center of the  $\beta$ -amyloid peptide region (Esch et al., 1990; Sisodia, 1992). The products yielded by this route are a large N-terminal region of

APP (APP $\alpha$ ) and a membrane associated C-terminal fragment (C83), which is subsequently hydrolysed by  $\gamma$ -secretase to yield the nearly unknown small p3 peptide. This is the non-amyloidogenic route because the cleavage site is located approximately in the middle of the A $\beta$  sequence, with no possibility of A $\beta$  formation.

- 5     The second APP processing pathway is the N- and C-terminal cleavage of APP by  $\beta$ - and  $\gamma$ -secretase (Fig. 1). The resulting molecules of these two proteolytic steps are the central fragments of APP, A $\beta$ <sub>40</sub> and A $\beta$ <sub>42</sub>, A $\beta$ <sub>40</sub> being the more abundant of the whole A $\beta$  formed (Conde, 2002).  $\beta$ -secretase cleaves at the amino terminus of the  $\beta$ -amyloid peptide and occurs first, followed by  $\gamma$ -secretase, which releases the carboxy terminus 10    of the peptide. This is based upon the observation that C-terminal fragments produced by  $\beta$ -secretase cleavage are readily apparent in cells, whereas APP fragments corresponding to a single C-terminal  $\gamma$  cleavage are not (Haass et al., 1992; Seubert et al., 1992).
- 15    Molecular heterogeneity of APP processing that generates A $\beta$  peptides results, amongst others, from different types of mutations in familial autosomic dominant Alzheimer's disease (FAD AD), located near the  $\beta$  or the  $\gamma$  cleavage sites (De Strooper, 2000). These different pathogenic mutations can be modeled in transgenic mice (Chen et al., 2000). However, AD is essentially non-familial, including more 20    than 99% of all patients, according to a large scale population study (Campion et al., 1999). An overexpression of A $\beta$  or a change in the ratio A $\beta$ <sub>42</sub>/A $\beta$ <sub>40</sub> is well demonstrated in FAD AD, but not in non-FAD AD, which amyloidosis is explained by a lack of A $\beta$  clearance or an increase of fibrillogenesis.
- 25    In the human brain, amyloidosis is observed first as diffuse aggregates of A $\beta$ <sub>42</sub> peptides, which accumulate progressively as amyloid plaques, followed by the deposition of A $\beta$ <sub>40</sub> peptides (Delacourte et al. 2001; 2002). A microglial cell proteolysis of A $\beta$ <sub>42</sub> into A $\beta$ <sub>40</sub> species has also been suggested (Fukumoto et al., 1996). The latter peptides are also observed in large quantities in the cerebral vessel walls, to 30    constitute amyloid angiopathy, which is found in variable amounts in AD brains (Barelli et al., 1997; Wisniewski et al., 1997). Senile plaques consist largely of

insoluble A $\beta$  surrounded by a variety of neuronal and glial processes. This amorphous, acellular material is found in the spaces between the brain's nerve cells.

Both tau pathology and A $\beta$  aggregation should be used as neuropathological  
5 diagnostic criteria for AD (consensus criteria for 1997). Clinical AD is diagnosed in patients with tau pathology in the frontal pole and the parietal cortex (stages 7 to 10) and the presence of A $\beta_{42}$  aggregates above 50  $\mu\text{g}/\text{gram}$  of wet tissue in these cortical areas. Infralclinical AD is diagnosed in non-demented patients or patients with mild cognitive impairment, with insoluble A $\beta_{42}$  at a concentration of 10  $\mu\text{g}/\text{gram}$  of tissue  
10 in neocortical areas, such as the frontal pole or the parietal cortex, and a tau pathology in the hippocampal area. In infralclinical AD patients, tau pathology can be asymptomatic up to stage 6. Non-demented patients can be considered as "pure controls" or "normal aging" (as far as tau and APP pathologies are concerned) if tau pathology is absent in all cortical areas, including the hippocampal area and if there is  
15 no trace of A $\beta_{42}$  aggregates in neocortical areas. If the patients are older than 75 years, very discrete or moderate tau pathology is likely to be found in the hippocampal area (stages 1 to 3), due to aging or a pathological process that remains to be determined. But these aged non-demented patients can be considered as controls as they have no detectable A $\beta_{42}$  aggregates (Delacourte et al., 1999; 2001).

20

A very significant effort is underway to test a large number of therapeutic options for AD. These approaches include numerous agents such as acetylcholinesterase inhibitors, nonsteroidal anti-inflammatory drugs (NSAIDS), estrogen, neurotrophic agents, and even vitamins (Sramek and Cutler, 2000; Thal, 2000). Three general sub-  
25 approaches have arisen with the ultimate goal of limiting the presence of A $\beta$ , in order to slow, stop or reverse the progression of AD. These approaches aim at preventing the formation of deposition of A $\beta$  and to clear the already formed A $\beta$ . The first way may be undertaken by enhancing the activity of  $\alpha$ -secretase or inhibiting  $\beta$ - and  $\gamma$ -secretases. Upregulation or inhibition of enzymes is however a delicate and uncertain task, certainly when their normal biological activity is not completely known. The second focus is avoiding the aggregation of the already formed A $\beta$  into fibrils. In this approach, however, the possibility that aggregation or fibrillogenesis inhibitors may reverse the process must be considered (Klein et al., 2001). The third route, and  
30

currently the most promising strategy for the treatment of AD, is the immunisation with A $\beta$  or some suitable fragment thereof to obtain antibody-mediated clearance of A $\beta$ . Various research teams have reported on the benefits of immunisation of different lines of APP transgenic mice with A $\beta$ . Immunization with A $\beta$  prevented the 5 development of  $\beta$ -amyloid plaque formation and removed existing plaques (Schenk et al., 1999; 2000). It further reduced the deposition of cerebral fibrillar A $\beta$ , the cerebral A $\beta$  burden and the cognitive dysfunction (Janus et al., 2000; Morgan et al., 2000; Weiner et al., 2000; Lemere et al., 2000a; Sigurdsson et al., 2001; Lemere et al., 2001). Even passive administration of antibodies alone was sufficient to reduce 10 plaque pathology (Bard et al., 2000; Bacskai et al., 2001; DeMattos et al., 2001) and reverse memory impairment (Dodart et al., 2002). Anti- A $\beta$  antibodies appear to bind to plaques and then, through what is likely an Fc-mediated phagocytosis process, the plaque material is taken up by microglia cells and hydrolyzed. Clearance of amyloid 15 deposits in the brain of AD patients using vaccination against A $\beta$  peptide is a novel approach that opens treatment perspectives (Schenk et al., 2001).

The above teachings, however, do not identify a specific chemical nature of the A $\beta$  peptide to be employed in vaccination strategies. The above teachings are restricted to the use of any of the naturally occurring forms of  $\beta$ -amyloid peptide (i.e. A $\beta$ <sub>39</sub>, A $\beta$ <sub>40</sub>, 20 A $\beta$ <sub>41</sub>, A $\beta$ <sub>42</sub> or A $\beta$ <sub>43</sub>). However, A $\beta$  is also a physiological product with an unknown function and vaccination with naturally occurring A $\beta$  could generate an undesirable immune reaction. In clinical Phase II trials with AN-1792, a synthetic form of A $\beta$ <sub>42</sub>, symptoms consistent with inflammation in the CNS were reported for 15-19 patients (Conde et al., 2002). Therefore, the efficiency of a therapeutic strategy relies on the 25 precise knowledge of the chemical nature of the first amyloid deposits that seed fibrillogenesis and that are pathological, rather than physiological, in order to avoid autoimmune responses. Only a clear understanding of the precise chemical nature of the pathological A $\beta$  species prone to aggregation and present in the very earliest deposits will enable the correct diagnosis of a person susceptible to or at risk of 30 developing a disease associated with  $\beta$ -amyloid formation and/or aggregation such as AD and thus enable the subsequent prevention of A $\beta$  aggregation or the subsequent removal of the amyloid burden by vaccination.

Since the discovery of amyloid-beta (A $\beta$ ) as the major constituent of amyloid deposits in Alzheimer's disease (AD) (Glenner and Wong, 1984), amino-truncated A $\beta$  peptides have been identified in plaques of AD patients (Masters et al., 1985). A modified A $\beta$  peptide, starting with pyroglutamyl residue at position 3 of the A $\beta$  sequence, was  
5 identified in A $\beta$  deposits of mainly Down's syndrome patients by Saido et al. (1995; 1996) and Harigaya et al. (2000). N-terminal degradations were observed in A $\beta$  peptide deposits from AD demented patients (Kalback et al., 2002). These studies, however, do not identify or differentiate between A $\beta$  C-terminal variants (A $\beta$ <sub>39</sub>, A $\beta$ <sub>40</sub>, A $\beta$ <sub>41</sub>, A $\beta$ <sub>42</sub> or A $\beta$ <sub>43</sub>). Furthermore, it is quite probable that these N-terminal  
10 truncations of A $\beta$  peptides are the result of artefacts of experimental procedures used to extract the A $\beta$  peptides of *in situ* amyloid deposits of AD patients (Masters et al., 1985; Kalback et al., 2002). They could also result from catabolism of amyloid deposits by a late stage proteolytic activity (generated by microglial cells for example) generated in AD brains (Kawarabayashi et al., 2001). N-terminal truncated A $\beta$   
15 peptides can also be produced by cell models transfected with a mutated amyloid protein precursor (APP) (Cescato et al., 2000) or when cells are treated with drugs such as baflomycin A1 or ammonium chloride (Haass et al., 1995; Schrader-Fischer and Paganetti, 1996). Here again, the dramatic physiological dysfunction that is induced, triggers a proteolytic activity, by membrane associated proteases. In  
20 summary, the above data suggest that in individuals with fully developed AD, amino-truncated A $\beta$  are components of the amyloid deposits. Their production could be a late event generated by microglial cells or by experimentally-induced proteolytic activities.  
  
25 For diagnosis, prevention and treatment of amyloidosis, however, it is mandatory to know the specific chemical nature of A $\beta$  peptides at the very early stages of amyloid deposition, before ongoing microglial or astrocytic reactions, generating proteolytic activity. At present, no data are available on the specific structure of pathological A $\beta$  peptides that are prone to aggregation and/or that are present in amyloid deposits that  
30 seed fibrillogenesis, and that are not yet subject to proteolysis mediated by microglial and astrocyte activity.

## SUMMARY OF THE INVENTION

The present invention provides compositions and methods for preventing and treating diseases characterized by  $\beta$ -amyloid formation and/or aggregation such as 5 Alzheimer's disease. The methods of the invention encompass the induction of an immune response against A $\beta$  peptides with a very specific chemical nature, i.e. N-terminal truncated and/or post-translationally modified A $\beta$  peptides. The invention thus relates to a preparation comprising a  $\beta$ -amyloid variant or a N-terminal fragment thereof characterized in that said  $\beta$ -amyloid variant or said N-terminal fragment 10 thereof contains a truncation and/or a post-translational modification at its N-terminus.

In a preferred embodiment, the present invention provides a preparation comprising a N-terminal truncated and/or post-translationally modified  $\beta$ -amyloid variant or a N-terminal fragment thereof, further characterized in that said N-terminal truncated  $\beta$ -amyloid variant or said N-terminal fragment thereof starts at position 2, 3, 4, 5, 6, 7, 8, 15 9 or 10 of  $\beta$ -amyloid and that the post-translational modification is a methylation or a pyroglutamylolation.

In another preferred embodiment, the present invention provides a preparation comprising a N-terminal truncated and/or post-translationally modified  $\beta$ -amyloid 20 variant or a N-terminal fragment thereof, further characterized in that the pyroglutamylation is present at position 3 of a N-terminal truncated  $\beta$ -amyloid variant starting at position 3 of  $\beta$ -amyloid.

In another preferred embodiment, the invention provides a preparation comprising a N-terminal truncated and/or post-translationally modified  $\beta$ -amyloid variant or a N-terminal fragment thereof, further characterized in that the  $\beta$ -amyloid variant or the N-terminal fragment thereof comprises an amino acid sequence selected from the group 25 consisting of SEQ ID NOs 1 to 165.

In another preferred embodiment of the invention, the present invention provides a preparation comprising a N-terminal truncated and/or post-translationally modified  $\beta$ -amyloid variant or a N-terminal fragments thereof, further characterized in that the  $\beta$ -amyloid variant or the N-terminal fragment thereof contains an additional 30 modification resulting in a separated spot of the  $\beta$ -amyloid variant or the N-terminal fragment thereof on two-dimensional gel electrophoresis compared to the spot

obtained with the  $\beta$ -amyloid variant or the N-terminal fragment thereof without said additional modification.

The finding of said N-terminal truncated and/or post-translationally modified A $\beta$  variants, which are a result of an aberrant pathway of APP metabolism, supports the presence of N-terminal APP soluble fragments containing extra amino acids at the C-terminal tail, resulting from truncated cleavage by  $\beta$  like secretase (i.e. behind amino acid 1, 2, 3, 4, 5, 6, 7, 8 or 9 of A $\beta$ ). Accordingly, the invention also relates to a preparation comprising a N-terminal APP soluble fragment obtainable by secretase cleavage of APP, characterized in that the C-terminal end of said N-terminal APP soluble fragment consists of position 1, 1 to 2, 1 to 3, 1 to 4, 1 to 5, 1 to 6, 1 to 7, 1 to 8 or 1 to 9 of  $\beta$ -amyloid. The invention also relates to a C-terminal fragment of such a N-terminal APP soluble fragment.

In a preferred embodiment, the present invention relates to a preparation comprising a N-terminal APP soluble fragment obtainable by secretase cleavage of APP, or a C-terminal fragment thereof, further characterized in that said N-terminal APP soluble fragment or said C-terminal fragment thereof comprises an amino acid sequence selected from the group consisting of SEQ ID NOs 1 to 6, 14 to 18, 27 to 30, 53 to 55, 66 to 67, 79, and 166 to 261.

20

The invention further relates to a nucleic acid preparation comprising a nucleic acid sequence capable of encoding the N-terminal truncated and/or post-translationally modified  $\beta$ -amyloid variant of the invention or the N-terminal fragment thereof.

The invention also relates to a nucleic acid preparation comprising a nucleic acid sequence capable of encoding the N-terminal APP soluble fragment of the invention or the C-terminal fragment thereof.

30 The invention further relates to a method for the preparation of an antibody that specifically recognizes a N-terminally truncated and/or post-translationally modified  $\beta$ -amyloid variant, comprising the following steps:

(a) Immunizing an animal with a preparation comprising a N-terminal truncated and/or post-translationally modified  $\beta$ -amyloid variant or a N-terminal fragment thereof or with a nucleic acid preparation encoding a N-terminal

truncated and/or post-translationally modified  $\beta$ -amyloid variant or a N-terminal fragment thereof;

- (b) Obtaining the antibodies generated by the immunization in step (a);
- (c) Screening the antibodies obtained in step (b) for their specific recognition of N-terminal truncated and/or post-translationally modified  $\beta$ -amyloid variant.

5 The invention also relates to an antibody obtainable by the above method.

The invention further also relates to a method for the preparation of an antibody that specifically recognizes a N-terminal APP soluble fragment of the invention, comprising the following steps:

- 10 (a) Immunizing an animal with a preparation of N-terminal APP soluble fragment of the invention or a C-terminal fragment thereof or with a nucleic acid preparation encoding a N-terminal APP soluble fragment of the invention or a C-terminal fragment thereof;
- (b) Obtaining the antibodies generated by the immunization in step (a);
- 15 (c) Screening the antibodies obtained in step (b) for their specific recognition of a N-terminal APP soluble fragment of the invention.

The invention also relates to an antibody obtainable by the above method.

As indicated above, the present invention provides preparations and methods for preventing and treating, in a mammal, a disease characterized by  $\beta$ -amyloid formation and/or aggregation. In accordance, the invention also relates to a vaccine composition or a therapeutic composition comprising a N-terminal truncated and/or post-translationally modified  $\beta$ -amyloid variant or a N-terminal fragment thereof, comprising an antibody recognizing said N-terminal truncated and/or post-translationally modified  $\beta$ -amyloid variant, or comprising a nucleic acid encoding said N-terminal truncated and/or post-translationally modified  $\beta$ -amyloid variant or said N-terminal fragment thereof.

25 The invention thus also relates to a method for the prevention and/or treatment, in an mammal, of a disease associated with  $\beta$ -amyloid formation and/or aggregation such as Alzheimer's disease, said method comprising the administration, to said mammal, of a vaccine composition or a therapeutic composition of the invention.

Another aspect of the present invention relates to a diagnostic or theranostic kit comprising a preparation of N-terminal truncated and/or post-translationally modified variant of the invention or a N-terminal fragment thereof, comprising a preparation of N-terminal APP soluble fragment of the invention or a C-terminal fragment thereof, or comprising an antibody recognizing said peptide or fragments.

The invention also relates to a method for the measurement, in a mammal, of the immune response induced by vaccination or therapeutic application with a vaccine composition or a therapeutic composition of the invention, said method comprising the following steps:

- 10 (a) Determining, in a sample obtained from said mammal, the amount of antibody specific for a N-terminal truncated and/or post-translationally modified  $\beta$ -amyloid variant of the invention;
- (b) Comparing the amount determined in step (a) with the amount of antibody specific for said N-terminal truncated and/or post-translationally modified  $\beta$ -amyloid variant present in the mammal before vaccination or therapeutic application with the vaccine or therapeutic composition of the invention;
- 15 (c) Concluding, from the comparison in step (b), whether the mammal is responding to the vaccination or therapy, an increased amount of antibody specific for said N-terminal truncated and/or post-translationally modified  $\beta$ -amyloid variant being an indication that the mammal is responding to the vaccination or therapy.

The invention further relates to a method for determining, in a mammal, the susceptibility to a disease associated with  $\beta$ -amyloid formation and/or aggregation such as Alzheimer's disease, for determining, in a mammal, the risk of developing a disease associated with  $\beta$ -amyloid formation and/or aggregation such as Alzheimer's disease, for screening of the clearance of  $\beta$ -amyloid deposition in a mammal, or for predicting the level of  $\beta$ -amyloid burden in a mammal, said method comprising the following steps:

- 20 (a) Determining, in said mammal, the amount of N-terminal truncated and/or post-translationally modified  $\beta$ -amyloid variant of the invention or the amount of N-terminal APP soluble fragment of the invention, or the amount of antibody specific for said  $\beta$ -amyloid variant or said N-terminal APP soluble fragment of the invention;

- (b) Comparing the amount determined in step (a) with the amount of said N-terminal truncated and/or post-translationally modified  $\beta$ -amyloid variant, said N-terminal APP soluble fragment or said antibody in a control mammal;
- 5 (c) Concluding, from the comparison in step (b), whether the mammal is susceptible to a disease associated with  $\beta$ -amyloid formation and/or aggregation such as Alzheimer's disease, whether the mammal is at risk of developing a disease associated with  $\beta$ -amyloid formation and/or aggregation such as Alzheimer's disease, whether the  $\beta$ -amyloid deposition in the mammal is cleared, or what the level of  $\beta$ -amyloid burden is in said mammal.
- 10 In a preferred embodiment, the present invention relates to a method as above, further characterized in that the amount of N-terminal truncated and/or post-translationally modified  $\beta$ -amyloid variant, of N-terminal APP soluble fragment or the amount of antibody specific for said  $\beta$ -amyloid variant or said APP soluble fragment is determined on a tissue sample taken from said mammal.
- 15 In a preferred embodiment, the present invention also relates to a method for predicting the level of  $\beta$ -amyloid burden in a mammal, said method comprising the following steps:
- 20 (a) Administration, to said mammal, of a vaccine composition or a therapeutic composition of the invention;
- (b) Determining, in a biological fluid sample obtained from said mammal, the amount of N-terminal truncated and/or post-translationally modified  $\beta$ -amyloid variant of the invention;
- 25 (c) Comparing the amount determined in step (a) with the amount of said N-terminal truncated and/or post-translationally modified  $\beta$ -amyloid variant in a biological fluid sample obtained from a control mammal;
- (d) Concluding, from the comparison in step (b), what the level of  $\beta$ -amyloid burden is in said mammal.

## **FIGURE LEGENDS**

**Figure 1.** Amino acid sequence of A $\beta$  with the  $\alpha$ -,  $\beta$ -, and  $\gamma$ -secretase cleavage sites in APP indicated.

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**Figure 2.** Two-dimensional electrophoretic analysis of A $\beta$  species present in brain obtained from Alzheimer's disease patients. A $\beta$  aggregates solubilized with formic acid were resolved by 2D gel electrophoresis. A $\beta$  monomers (4kDa) and dimers (8kDa) were labelled with antibodies WO2 and 6E10 against the amino-terminal region of A $\beta$  (N-ter(5-8) and N-ter(4-13) panels, respectively). The carboxy-terminal tails of A $\beta_{42}$  and A $\beta_{40}$  were detected with 21F12 and ADA40, respectively (A $\beta$ -42 and A $\beta$ -40 panels). The major A $\beta$  species recovered with our extraction method were stained with Coomassie Blue, and 10 spots were subsequently analysed by mass spectrometry (Table 3). The results presented were obtained from the AD patient showing the largest quantity of amyloid deposits. The isoelectric points (pI) and the A $\beta$  spots used for mass spectrometric analysis are indicated. Note that dimeric species of A $\beta$  are not stained by Coomassie Blue.

**Figure 3.** Aggregated A $\beta$  species present in brain obtained from infraclinical AD

20 patients. Formic acid solubilized A $\beta$  species derived from the brain tissue of non-demented patients were resolved by 2D gel electrophoresis. A $\beta_{40}$  species are not detected with our ADA40 antiserum. 21F12 detects both A $\beta_{42}$  monomers (4 kDa staining) and dimers (8 kDa staining). Both WO2 (panel N-ter (5-8)) and 6E10 (panel N-ter (4-13)) antibodies stained a single A $\beta$  peptide spot with pI 5.30.

25

**Figure 4.** Dimers of A $\beta$  are essentially composed of amino-truncated A $\beta$  peptides.

The brain tissue of a control individual (Ctrl, S0), three non-demented cases (S1, S2 and S6) and one AD case (S10) were lysed in formic acid. According to the nomenclatures defined by Delacourte et al. (2002) and Braak and Braak (1991), the stages of tau pathology (S0 to S10) and the amyloid staging classification (B or C) are indicated at the top of the lanes, respectively. Ten ng of A $\beta$  peptides 1-40 and 1-42 were loaded in parallel (first and second lane). A $\beta$  x-42 was identified with 21F12

(panel A $\beta$ -42) and the amino-terminal region with WO2 (WO2 panel). Molecular weights are indicated on the left, and an arrow indicates the amino-truncated variants labelled by 21F12. Note that the same AD case was used for both 2D electrophoretic analysis and mass spectrometry.

## DETAILED DESCRIPTION OF THE INVENTION

### N-terminal truncated and/or post-translation modified A<sub>β</sub> peptides

The present invention provides compositions and methods for preventing and treating diseases characterized by β-amyloid formation and/or aggregation such as Alzheimer's disease. The methods of the invention encompass the induction of an immune response against A<sub>β</sub> peptides with a very specific chemical nature, i.e. N-terminal truncated and/or post-translationally modified A<sub>β</sub> peptides. N-terminal truncated and/or post-translationally modified A<sub>β</sub> variants are the aggregated species of A<sub>β</sub> that seed amyloid deposition at the infraclinical stages of Alzheimer pathology. They were identified, for the first time, in the brain tissue of non-demented individuals with both neurofibrillary degeneration and amyloid deposition at low levels, while they were not found in "pure control" individuals without neurofibrillary degeneration or amyloid deposition. Hence, these N-terminal truncated and/or post-translationally modified A<sub>β</sub> variants correspond to the infraclinical stages of AD (i.e. the earliest possible stages I and II of AD pathology, as described by Braak and Braak, 1991 or the Tau pathology stages 1 to 6 according to Delacourte et al., 1999) and form the seeds of amyloid deposition. These N-terminal truncated and/or post-translationally modified A<sub>β</sub> variants were observed in the brain tissue not yet affected by microglial or astrocyte reaction that generate proteolytic activities. This indicates that these N-terminal truncated and/or post-translationally modified A<sub>β</sub> variants are not the result of late stage proteolytic activity but that they should be *in situ* products, resulting from an aberrant pathway of APP metabolism. These N-terminal truncated and/or post-translationally modified A<sub>β</sub> variants can thus be considered as the very first pathological species related to the early events of amyloidosis.

A<sub>β</sub> peptide, A<sub>β</sub>, β-amyloid peptide, or A4 peptide are used interchangeable throughout the present invention and refer to a peptide of 39-43 amino acids (A<sub>β</sub><sub>39</sub>, A<sub>β</sub><sub>40</sub>, A<sub>β</sub><sub>41</sub>, A<sub>β</sub><sub>42</sub> or A<sub>β</sub><sub>43</sub>), which is the principal component of characteristic plaques of Alzheimer's disease. A<sub>β</sub> is generated by processing of a larger protein APP by two enzymes, termed β- and γ-secretases (Haass et al. 1992; Seubert et al. 1992). The sequences of the A<sub>β</sub><sub>42</sub> peptide is the following:

DAEFRHDSGYEVHHQLVFFAEDVGSNKGAIIGLMVGGVIA  
(SEQ ID NO 144)

A $\beta$ <sub>41</sub>, A $\beta$ <sub>40</sub> and A $\beta$ <sub>39</sub> differ from A $\beta$ <sub>42</sub> by the omission of Ala (A), Ile-Ala (IA), and  
5 Val-Ile-Ala (VIA) respectively from the C-terminal end. A $\beta$ <sub>43</sub> differs from A $\beta$ <sub>42</sub> by  
the presence of a threonine residue at the C-terminus.

Diseases associated with  $\beta$ -amyloid formation and/or aggregation are diseases in  
which the  $\beta$ -amyloid is formed in the brain as a result of  $\beta$ - and  $\gamma$ -cleavage of APP  
10 and/or in which said  $\beta$ -amyloid forms oligomers. Diseases associated with  $\beta$ -amyloid  
formation and/or aggregation include but are not limited to Disease with Lewy bodies  
(DLB) with amyloid, Down's syndrome, Alzheimer's disease, both late and early  
onset.

15 Disaggregated or monomeric A $\beta$ , as used in the present invention, means soluble,  
monomeric peptide units of A $\beta$ . Aggregated A $\beta$  is a mixture of oligomers in which  
the monomeric units are held together by non-covalent bonds. "Disaggregating" refers  
to solubilization of aggregated proteins typically held together by non-covalent  
bounds.  $\beta$ -amyloid deposits comprise a A $\beta$  peptide aggregated to an insoluble mass.  
20 In most cases, the aggregate has a  $\beta$ -pleated sheet structure. The present invention  
demonstrates that A $\beta$  dimers, the very first aggregates in A $\beta$  deposition, are mainly or  
exclusively composed of N-terminal truncated and/or post-translationally modified  
A $\beta$  variants. This supports that the basis mechanisms of  $\beta$ -amyloidosis are related to  
an aggregation of these N-terminal truncated and/or post-translationally modified A $\beta$   
25 variants.

The present invention thus relates to a preparation comprising a  $\beta$ -amyloid variant or  
a N-terminal fragment thereof characterized in that said  $\beta$ -amyloid variant or said N-  
terminal fragment thereof contains a truncation and/or a post-translational  
30 modification at its N-terminus. These  $\beta$ -amyloid variants and the N-terminal fragment  
thereof are jointly called "N-terminal truncated and/or post-translationally modified  
A $\beta$  peptides."

The preparation of the invention is typically substantially pure. This means that the  $\beta$ -amyloid variant or the N-terminal fragment thereof is typically at least about 50% w/w (weight/weight) pure, as well as being substantially free from interfering proteins and contaminants. It is preferred that the  $\beta$ -amyloid variant or the N-terminal fragment thereof is at least about 80% w/w and, more preferably at least 90% or about 95% w/w pure. Using conventional protein purification techniques, homogeneous peptides of at least 99% w/w can even be obtained.

5 The N-terminal truncated and/or post-translationally modified  $\beta$ -amyloid variants that were present in brains from patients in the infraclinical stages of Alzheimer's disease typically consisted of A $\beta$  starting at position 2, 3, 4, 5, 6, 7, 8, 9 or 10. Post-translational modifications that were characterized are methylation or pyroglutamylation. But N-terminal truncated A $\beta$  variants without any post-translational modification have also been identified. In accordance, the present 15 invention relates to the preparation as described above, further characterized in that the N-terminal truncated  $\beta$ -amyloid variant or the N-terminal fragment thereof starts at position 2, 3, 4, 5, 6, 7, 8, 9 or 10 of  $\beta$ -amyloid and that the post-translational modification is a methylation or a pyroglutamylation.

In a preferred embodiment of the invention the  $\beta$ -amyloid variant or the N-terminal 20 fragment thereof starts at position 2, 3, 4, 5, 8, 9 or 10 of  $\beta$ -amyloid. In another preferred embodiment, the  $\beta$ -amyloid variant or the N-terminal fragment thereof starts at position 3, 4, 5, 8 or 9 of  $\beta$ -amyloid. In another preferred embodiment, the  $\beta$ -amyloid variant or the N-terminal fragment thereof contains a methylation at any one of positions 1, 2, 4 or 6. In another preferred embodiment, the  $\beta$ -amyloid variant or 25 the N-terminal fragment thereof is pyroglutamylated at position 3. In accordance, the present invention relates to the preparation as discussed above, further characterized in that the pyroglutamylation is present at position 3 of a N-terminal truncated  $\beta$ -amyloid variant starting at position 3 of  $\beta$ -amyloid.

30 The N-terminal fragments of the invention typically have a sequence of at least 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 20 or more contiguous amino acids from the N-terminal part of the N-terminal truncated and/or post-translationally modified  $\beta$ -amyloid variant. In accordance, the N-terminal fragments of the invention may consist

of or comprise the peptides as indicated in SEQ ID NOs 1 to 143. In other cases, the β-amylod peptides of the invention may consist of longer polypeptides that include the β-amylod variant together with other amino acids. In accordance, the β-amylod peptides of the invention may also consist of or comprise the peptides as indicated in  
5 SEQ ID NOs 144 to 165. The present invention thus relates to the above preparation, further characterized in that the β-amylod variant or the N-terminal fragment thereof comprises an amino acid sequence selected from the group consisting of SEQ ID NOs 1 to 165.

In addition to a methylation or pyroglutamylation, the β-amylod peptides of the  
10 present invention may comprise other modifications. Therefore, the present invention also relates to the preparation as discussed above, further characterized in that the β-amylod variant or the N-terminal fragment thereof contains an additional modification resulting in a separated spot of the β-amylod variant or the N-terminal fragment thereof on two-dimensional gel electrophoresis compared to the spot obtained with the β-amylod variant or the N-terminal fragment thereof without said  
15 additional modification. Additional modifications include any modification which induces an apparent shift in the isoelectric point (pI) and/or molecular weight (MW) on a two dimensional gel. They include but are not limited to phosphorylation, addition of isoaspartate, acetylation, glycosylation, racemization, isomerization, proteolysis,  
20 stereomerization, cyclization. In a preferred embodiment, the additional modification is an isoaspartate on position 7 of the N-terminal truncated Aβ peptide.

The demonstration, by the present inventors, that N-terminal truncated and/or post-translationally modified Aβ variants are amongst the earliest pathological antigens of  
25 Alzheimer disease demonstrates that they are the ideal target for vaccination. Indeed, N-terminal truncated and/or post-translationally modified peptides will target the immune response specifically towards these N-terminal truncated and/or post-translationally modified Aβ variants, which do not exist naturally. The resulting immune response will, consequently, selectively clear these pathological N-terminal  
30 truncated and/or post-translationally modified Aβ variants.

In order to induce an immune response, the preparation of the invention should be immunogenic. An “immunogenic preparation” is a preparation that comprises an

“immunogenic agent” or “immunogen” that is capable of inducing an immunological response directed against itself upon administration to a recipient mammal, optionally in conjunction with an adjuvant. The immunogenic preparation of the present invention may comprise a N-terminal truncated and/or post-translational modified A $\beta$  peptide as immunogen. The term “immunological” or “immune” response refers to the development of a beneficial humoral (antibody mediated) and/or a cellular (mediated by antigen-specific T-cells or their secretion products) response directed against the N-terminal truncated and/or post-translationally modified A $\beta$  peptide in a recipient mammal. Such a response can be an active response induced by administration of immunogen or a passive response induced by administration of antibody or primed T-cells (see further). A cellular immune response is elicited by the presentation of polypeptide epitopes in association with Class I or Class II MHC molecules to activated antigen-specific CD4 T helper cells and/or CD8+ cytotoxic T-cells. The response may also involve activation of monocytes, macrophages, NK cells, basophils, dendritic cells, astrocytes, microglia cells, eosinophils or components of innate immunity.

Some agents for inducing an immune response contain the appropriate epitope for induction of an immune response against amyloid deposits but are in themselves too small to be immunogenic. In this situation, a peptide immunogen can be linked to a suitable carrier to help elicit an immune response. Suitable carriers include serum albumins, keyhole limpet hemocyanin, immunoglobulin molecules, thyroglobulin, ovalbumin, tetanus toxoid, or a toxoid from other pathogenic bacteria, such as diphtheria, *E. coli*, cholera, or *H. pylori*, or an attenuated toxin derivative. Other carriers for stimulating or enhancing an immune response include cytokines such as IL-1, IL-1  $\alpha$  and  $\beta$  peptides, IL-2,  $\alpha$ TNF, IL-10, GM-CSF, and chemokines, such as MIP $\alpha$  and  $\beta$  and RANTES. Immunogenic agents can also be linked to peptides that enhance transport across tissues, as described in WO 97/17613 and WO 97/17614. Immunogenic agents can be linked to carriers by chemical crosslinking. Techniques for linking an immunogen to a carrier include the formation of disulfide linkages using N- succinimidyl-3-(2-pyridyl-thio) propionate (SPDP) and succinimidyl 4-(N-maleimidomethyl)cyclohexane-1-carboxylate (SMCC) (if the peptide lacks a sulphydryl group, this can be provided by addition of a cysteine residue). These reagents create a disulfide linkage between themselves and peptide cysteine residus on

one protein, and an amide linkage through the  $\varepsilon$ -amino on a lysine, or other free amino group in other amino acids. A variety of such disulfide/amide-forming agents are described by Janssen et al. (1982). Other bifunctional coupling agents form a thioether rather than a disulfide linkage. Many of these thio-ether-forming agents are 5 commercially available and include reactive esters of 6-maleimidocaproic acid, 2-bromoacetic acid, and 2-iodoacetic acid, 4-(N-maleimido-methyl)cyclohexane-1-carboxylic acid. The carboxyl groups can be activated by combining them with succinimide or 1-hydroxyl-2-nitro-4-sulfonic acid, sodium salt. Immunogenic peptides can also be expressed as fusion proteins with carriers. The immunogenic 10 peptide can be linked at the amino terminus, the carboxyl terminus, or internally to the carrier. Optionally, multiple repeats of the immunogenic peptide can be present in the fusion protein.

#### N-terminal APP soluble fragments

15 The finding of the N-terminal truncated and/or post-translationally modified A $\beta$  variants as a result from an aberrant pathway of APP metabolism supports the presence of N-terminal APP soluble fragments containing extra amino acids at the C-terminal tail, resulting from truncated cleavage by  $\beta$  like secretase (i.e. behind amino acid 1, 2, 3, 4, 5, 6, 7, 8 or 9 of A $\beta$ ). As discussed in the background art section, A $\beta$  is 20 metabolized from APP by N- and C-terminal cleavage of APP by  $\beta$ - and  $\gamma$ -secretase (Fig. 1). APP is found in three isoforms, APP695, APP751, and APP770, referring, respectively, to the 695, 751, and 770 amino acid residue long polypeptides encoded by the human APP gene (Kang et al., 1987; Ponte et al. 1988; Kitaguchi et al., 1988). In the present invention, amino acids within the human amyloid precursor protein 25 (APP) are assigned numbers according to the sequence of the APP770 isoform (Fig. 1).  $\beta$ -secretase cleaves at the amino terminus of the  $\beta$ -amyloid peptide between positions 671 and 672 of APP. An aberrant cleavage of this  $\beta$ -secretase, however, more towards the A $\beta$  sequence, will result in N-terminal APP fragments with one or more additional A $\beta$  amino acids. In accordance, the present invention relates to a 30 preparation comprising a N-terminal APP soluble fragment obtainable by  $\beta$  like secretase cleavage of APP, characterized in that the C-terminal end of said N-terminal APP soluble fragment consists of position 1, 1 to 2, 1 to 3, 1 to 4, 1 to 5, 1 to 6, 1 to 7, 1 to 8 or 1 to 9 of  $\beta$ -amyloid. The invention also relates to a C-terminal fragment of

such a N-terminal APP soluble fragment. In a specific embodiment, the invention relates to the preparation as described above, further characterized in that the N-terminal APP soluble fragment or the C-terminal fragment thereof comprises an amino acid sequence selected from the group consisting of SEQ ID NOs 1 to 6, 14 to  
5 18, 27 to 30, 52 to 55, 66 to 67, 79, and 166 to 261.

The A $\beta$  variants of the invention, their N-terminal fragments, the N-terminal APP soluble fragments of the invention and the C-terminal fragments thereof can be synthesized by solid phase peptide synthesis or recombinant expression, or can be  
10 obtained from natural sources. Automatic peptide synthesizers are commercially available from numerous suppliers, such as Applied Biosystems (Foster City, California, US). Recombinant expression can be in bacteria, such as *E. coli*, in yeast, in insect cells or in mammalian cells. Procedures for recombinant expression are described by Sambrook et al. (1989).

15

Also included in the present invention are peptides and molecule, also called peptidomimetics, which retain the essential three dimensional shape and immunological binding capacity of the A $\beta$  peptides and APP soluble fragments of the invention. These peptidomimetics can mimic the A $\beta$  peptides and APP soluble  
20 fragments of the invention for inducing an immune response in a mammal recipient or for the formation of an immunological complex with an antibody specific for said A $\beta$  peptide or APP soluble fragment of the invention.

#### Nucleic acids

25 The present invention also relates to a nucleic acid preparation comprising a nucleic acid sequence capable of encoding the  $\beta$ -amyloid variant of the invention or the N-terminal fragment thereof or capable of encoding the N-terminal APP soluble fragment of the invention or the C-terminal fragment thereof. The nucleic acids of the invention can be DNA or RNA. A DNA molecule is "capable of expressing" a  
30 polypeptide, such as the the  $\beta$ -amyloid variant of the invention or the N-terminal fragment thereof or the N-terminal APP soluble fragment of the invention or the C-terminal fragment thereof, if it contains nucleotide sequences which contain transcriptional and translational regulatory information, and such sequences are

“operably linked” to nucleotide sequences which encode the polypeptide. An operably linkage is a linkage in which the regulatory DNA sequences and the DNA sequence sought to be expressed are connected in such a way as to permit gene expression. The regulatory regions needed for gene expression, in general, include a promoter region  
5 as well as the DNA sequences which, when transcribed into RNA, will signal the initiation of protein synthesis. Such regions will normally include those 5' -non-coding sequences involved in initiation of transcription and translation. A promoter region would be operably linked to a DNA sequence if the promoter were capable of effecting transcription of that DNA sequence. The nucleic acid segment encoding the  
10  $\beta$ -amyloid variant of the invention or the N-terminal fragment thereof or the N-terminal APP soluble fragment of the invention or the C-terminal fragment thereof should thus be linked to regulatory elements, such as a promoter and enhancer, that allow expression of the nucleic acid segment in the intended host. For expression in blood cells, for example, as is desirable for induction of an immune response in a mammal, promoter and enhancer elements from light or heavy chain immunoglobulin genes or the CMV major intermediate early promoter and enhancer are suitable to direct expression.  
15

The linked regulatory elements and coding sequences are often cloned into a vector. A vector is a nucleic acid, preferably a DNA molecule, capable of autonomous replication in a cell, to which a nucleic acid segment (e. g. a gene or polynucleotide, preferably DNA) can be operatively linked so as to bring about replication of the attached segment. Vectors capable of directing the expression of nucleic acid (preferably DNA) segments (genes) encoding for one or more proteins are referred to as “expression vectors”. An expression vector thus is any plasmid or virus into which  
20 a foreign nucleotide sequence (preferably DNA) may be inserted or expressed. A number of viral vector systems are available including (but not limited to) retroviral systems (Lawrie and Tumin, 1993), adenoviral vectors (Bett et al., 1993), adeno-associated virus vectors (Zhou et al., 1994), viral vectors from the pox family including vaccinia virus and the avian pox viruses, viral vectors from the alpha virus  
25 genus such as those derived from Sindbis and Semliki Forest Viruses (Dubensky et al., 1996), and papillomaviruses (Ohe et al., 1995; WO 94/12629; Xiao and Brandsma, 1996).

### Antibodies

In another embodiment, the invention relates to a method for the preparation of an antibody that specifically recognizes the N-terminal truncated and/or post-translationally modified  $\beta$ -amyloid variant of the invention. Said method comprises the following steps:

- (a) Immunizing an animal with an immunogenic preparation comprising a N-terminal truncated and/or post-translationally modified A $\beta$  peptide or with a nucleic acid preparation encoding said N-terminal truncated and/or post-translationally modified A $\beta$  peptide;
  - (b) Obtaining the antibodies generated by the immunization in step (a);
  - (c) Screening the antibodies obtained in step (b) for their specific recognition of N-terminal truncated and/or post-translationally modified  $\beta$ -amyloid variants.
- 15 The invention further relates to antibodies obtainable by the above method.

In a further embodiment, the present invention relates to a method for the preparation of an antibody that specifically recognizes the N-terminal APP soluble fragment of the invention. Said method comprises the following steps:

- (a) Immunizing an animal with a preparation of N-terminal APP soluble fragment of the invention or C-terminal fragment thereof or with the nucleic acid preparation encoding said N-terminal APP soluble fragment of the invention or said C-terminal fragment thereof;
  - (b) Obtaining the antibodies generated by the immunization in step (a);
  - (c) Screening the antibodies obtained in step (b) for their specific recognition of N-terminal APP soluble fragment of the invention.
- 25 The invention further relates to antibodies obtainable by the above method.

The term "specifical recognition", "specifically binding with", "specifically reacting with" or "specifically forming an immunological reaction with" refers to a binding reaction by the antibody to the N-terminal truncated and/or post-translationally modified  $\beta$ -amyloid variant or to the N-terminal APP soluble fragment respectively, which is determinative of the presence of the N-terminal truncated and/or post-

translationally modified β-amyloid variant or the N-terminal APP soluble fragment respectively in the sample tested, in the presence of a heterogeneous population of other proteins and/or other biologics. Thus, under the designated immunassay conditions, the specified antibody preferentially binds to a N-terminal truncated and/or post-translationally modified β-amyloid variant or to a N-terminal APP soluble fragment of the invention while binding to other proteins or protein isoforms does not occur in significant amounts. In a preferred embodiment the specified antibody preferentially binds to a N-terminal truncated and/or post-translationally modified β-amyloid variant or to a N-terminal APP soluble fragment of the invention while binding to normal, non pathological A $\beta$  or to normal N-terminal APP fragment does not occur in significant amounts.

As used herein, an “antibody” refers to a protein consisting of one or more polypeptides substantially encoded by immunoglobulin genes or fragments of immunoglobulin genes. The recognized immunoglobulin genes include the kappa, lambda, alpha, gamma, delta, epsilon and mu constant region genes, as well as the myriad immunoglobulin variable region genes. Light chains are classified as either kappa or lambda. Heavy chains are classified as gamma, mu, alpha, delta, or epsilon, which in turn define the immunoglobulin classes, IgG, IgM, IgA, IgD and IgE, respectively. The basic immunoglobulin (antibody) structural unit is known to comprise a tetramer or dimer. Each tetramer is composed of two identical pairs of polypeptide chains, each pair having one “light” (about 25 kD) and one “heavy” chain (about 50-70 kD). The N-terminus of each chain defines a variable region of about 100 to 110 or more amino acids, primarily responsible for antigen recognition. The terms “variable light chain (V<sub>L</sub>)” and “variable heavy chain (V<sub>H</sub>)” refer to these variable regions of the light and heavy chains respectively. Optionally, the antibody or the immunological portion of the antibody, can be chemically conjugated to, or expressed as, a fusion protein with other proteins.

Antibodies of the invention include, but are not limited to polyclonal, monoclonal, bispecific, human, humanized or chimeric antibodies, single variable fragments (ssFv), single chain fragments (scFv), Fab fragments, F(ab') fragments, fragments produced by a Fab expression library, anti-idiotypic antibodies and epitope-binding fragments of any of the above, provided that they retain the original binding

properties. Also mini-antibodies and multivalent antibodies such as diabodies, triabodies, tetravalent antibodies and peptabodies can be used in a method of the invention. The preparation and use of these fragments and multivalent antibodies has been described extensively in International Patent Application WO 98/29442. The 5 immunoglobulin molecules of the invention can be of any class (i.e. IgG, IgE, IgM, IgD and IgA) or subclass of immunoglobulin molecule.

The N-terminal truncated and/or post-translationally modified A $\beta$  variants, the N-terminal fragments thereof, the N-terminal APP soluble fragments of the invention or 10 the C-terminal fragments thereof can be used as an immunogen to generate the antibodies of the invention which specifically bind such an immunogen. Various host animals can be immunized for injection with said N-terminal truncated and/or post-translationally modified A $\beta$  variants, the N-terminal fragments thereof, the N-terminal APP soluble fragments of the invention or the C-terminal fragments thereof, including 15 but not limited to rabbits, mice, rats, etc. Various adjuvants may be used to enhance the immunological response, depending on the host species, including but not limited to complete or incomplete Freund's adjuvant, a mineral gel such as aluminum hydroxide, surface active substances such as lysolecithin, pluronic polyol, a polyanion, a peptide, an oil emulsion, keyhole limpet hemocyanin, dinitrophenol, or 20 an adjuvant such as BCG (bacille Calmette-Guerin) or corynebacterium parvum. For the preparation of monoclonal antibodies, any technique which provides for the production of antibody molecules by continuous cell lines in culture may be used. Hyperimmunization of an appropriate donor, generally a mouse, with the antigen is undertaken. Isolation of splenic antibody producing cells is then carried out. These 25 cells are fused to a cell characterized by immortality, such as a myeloma cell, to provide a fused cell hybrid (Hybridoma) which can be maintained in culture and which secretes the required monoclonal antibody. The cells are then cultured in bulk and the monoclonal antibodies harvested from the culture media for use. Specific techniques include but are not limited to the hybridoma technique developed by 30 Kohler and Milstein (1975), the human B-cell hybridoma technique (Kozbor et al., 1983) or the EBV-hybridoma technique to produce human monoclonal antibodies (Cole et al., 1985). Screening for the desired antibody can be done by techniques known in the art such as ELISA. For selection of an antibody that specifically binds a

N-terminal truncated and/or post-translationally modified A $\beta$  variant or a N-terminal APP soluble fragment of the invention, but that does not specifically bind another protein, normal A $\beta$  peptide or normal N-terminal APP soluble fragment, one can select on the basis of positive binding to the first and the lack of binding to the  
5 second. Thus, in a particular embodiment, the present invention provides an antibody that binds with greater affinity (particularly at least 2-fold, more particularly at least 5-fold, still more particularly at least 10-fold greater affinity) to a N-terminal truncated and/or post-translationally modified A $\beta$  variant or to a N-terminal APP soluble of the invention than to another protein. In another preferred embodiment, the  
10 present invention provides an antibody that binds with greater affinity (particularly at least 2-fold, more particularly at least 5-fold, still more particularly at least 10-fold greater affinity) to a N-terminal truncated and/or post-translationally modified A $\beta$  variant or to a N-terminal APP soluble fragment of the invention than to normal A $\beta$  peptide or to normal N-terminal APP soluble fragment.

15 While various antibody fragments are defined in terms of enzymatic digestion of an intact antibody with papain, pepsin or other proteases, one of skill will appreciate that such antibody fragments as well as full size antibodies may be synthesized de novo either chemically or by utilizing recombinant DNA methodology. Thus, the term  
20 antibody, as used herein also includes antibodies and antibody fragments either produced by the modification of whole antibodies or synthesized de novo using recombinant DNA methodologies.

25 The term "humanized antibody" means that at least a portion of the framework regions of an immunoglobulin is derived from human immunoglobulin sequences. The humanized versions of the mouse monoclonal antibodies can, for example, be made by means of recombinant DNA technology, departing from the mouse and/or human genomic DNA sequences coding for H and L chains or from cDNA clones coding for H and L chains. Humanized forms of mouse antibodies can be generated by linking the  
30 CDR regions of non-human antibodies to human constant regions by recombinant DNA techniques (Queen et al., 1989; WO 90/07861). Alternatively the monoclonal antibodies used in the method of the invention may be human monoclonal antibodies. Human antibodies can be obtained, for example, using phage-display methods (WO

91/17271; WO 92/01047). In these methods, libraries of phage are produced in which members display different antibodies on their outsersurfaces. Antibodies are usually displayed as Fv or Fab fragments. Phage displaying antibodies with a desired specificity are selected by affinity enrichment to N-terminal truncated and/or post-translationally modified A $\beta$  peptide or APP soluble fragment of the invention (or C-terminal fragment thereof). Human antibodies against N-terminal truncated and/or post-translationally modified A $\beta$  peptide or against APP soluble fragment can also be produced from non-human transgenic mammals having transgenes encoding at least a segment of the human immunoglobulin locus and an inactivated endogenous immunoglobulin locus (WO93/12227; WO 91/10741). Human antibodies can be selected by competitive binding experiments, or otherwise, to have the same epitope specificity as a particular mouse antibody. Such antibodies are particularly likely to share the useful functional properties of the mouse antibodies. Human polyclonal antibodies can also be provided in the form of serum from humans immunized with an immunogenic agent. Optionally, such polyclonal antibodies can be concentrated by affinity purification using N-terminal truncated and/or post-translationally modified A $\beta$  peptide or using N-terminal APP soluble fragment or a C-terminal fragment thereof as an affinity reagent. Monoclonal antibodies can be obtained from serum according to the technique described in WO 99/60846.

20

#### Vaccine and therapeutic compositions

As indicated above, the present invention provides preparations and methods for preventing and treating, in a mammal, a disease characterized by  $\beta$ -amyloid formation and/or aggregation by induction of an immune response in said mammal. In accordance, the present invention also provides a vaccine composition or a therapeutic composition comprising a N-terminal truncated and/or post-translationally modified A $\beta$  peptide as referred to above, comprising an antibody or T-cell specific for a N-terminal truncated and/or post-translationally modified A $\beta$  peptide as referred to above, or comprising a nucleic acid encoding a N-terminal truncated and/or post-translationally modified A $\beta$  peptide as referred to above.

As used herein, the term "preventing a disease" means inhibiting or reversing the onset of the disease, inhibiting or reversing the initial signs of the disease (i.e. formation and/or aggregation of A $\beta$  variants), inhibiting the appearance of clinical

symptoms of the disease. As used herein, the term “treating a disease” includes substantially inhibiting the disease, substantially slowing or reversing the progression of the disease, substantially ameliorating clinical symptoms of the disease or substantially preventing the appearance of clinical symptoms of the disease.

- 5 The mammal examined in the present invention may be a non-human mammal, such as (but not limited to) a cow, a pig, a sheep, a goat, a horse, a monkey, a rabbit, a hare, a dog, a cat, a mouse, a rat, an elk, a deer or a tiger. In a preferred embodiment, the mammal is a primate. In another preferred embodiment the mammal is a human, more preferably the mammal is a human adult.
- 10 The vaccine or therapeutic compositions of the present invention induce an immune response against the specific N-terminal truncated and/or post-translationally modified A<sub>β</sub> peptides of the invention. The induction of an immune response is “active” when an immunogen is administered to induce antibodies or T- cells reactive against the immunogen. The induction of an immune response is “passive” when an antibody is administered that itself binds to the N-terminal truncated and/or post-translationally modified A<sub>β</sub> variants in the mammal.  
In accordance, the vaccine or therapeutic compositions of the invention may also comprise antibodies that specifically bind to the N-terminal truncated and/or post-translationally modified A<sub>β</sub> variants of the invention.
- 15
- 20 Furthermore, immune responses against the N-terminal truncated and/or post-translationally modified A<sub>β</sub> peptides of the invention can also be induced by administration of nucleic acids encoding said N-terminal truncated and/or post-translationally modified A<sub>β</sub> peptides, or encoding recombinant antibodies that specifically recognize said N-terminal truncated and/or post-translational modified A<sub>β</sub> peptides. Such nucleic acids can be DNA or RNA. In order to facilitate the introduction of a recombinant DNA molecule into cells of the CNS, a number of different means for gene delivery can be used in association with the recombinant DNA molecule. The term “means for gene delivery” is meant to include any technique suitable for delivery of DNA molecules across the blood brain barrier and/or for transmembrane delivery across cell membranes. Non-limiting examples of means for gene delivery are viral vectors (e.g., adeno-associated virus-based vectors, lipids/liposomes, ligands for cell surface receptors, etc). DNA encoding an immunogen, or a vector containing the same, can be packaged into liposomes.
- 25
- 30

Suitable lipids and related analogs are described by US 5,208,036, 5,264,618, 5,279,833 and 5,283,185. Vectors and DNA encoding an immunogen can also be adsorbed to or associated with particulate carriers, examples of which include polymethyl methacrylate polymers and polylactides and poly(lactide-co-glycolides)

5 (McGee et al., 1996). Gene therapy vectors or naked DNA can be delivered *in vivo* by administration to an individual patient, typically by systemic administration (e.g., intravenous, intraperitoneal, nasal, gastric, intradermal, intramuscular, subdermal, or intracranial infusion) or topical application (see e.g., US 5,399,346). DNA can also be administered using a gene gun (Xiao and Brandsma, 1996). The DNA encoding an

10 immunogen is precipitated onto the surface of microscopic metal beads. The microprojectiles are accelerated with a shock wave or expanding helium gas, and penetrate tissues to a depth of several cell layers. For example, The Accel<sup>TM</sup> Gene Delivery Device manufactured by Agacetus, Inc. (Middleton WI, US) is suitable. Alternatively, naked DNA can pass through skin into the blood stream simply by

15 spotting the DNA onto skin with chemical or mechanical irritation (WO 95/05853). In a further variation, vectors encoding the N-terminal truncated and/or post-translationally modified peptide, or encoding recombinant antibodies specifically recognizing said N-terminal truncated and/or post-translationally modified peptide, can be delivered to cells *ex vivo*, such as cells explanted from an individual patient (e.g.,

20 lymphocytes, bone marrow aspirates, tissue biopsy) or universal donor hematopoietic stem cells, followed by reimplantation of the cells into a patient, usually after selection for cells which have incorporated the vector.

In a further variation, the N-terminal truncated and/or post-translationally modified A<sub>β</sub> peptide can be presented as a viral or bacterial vaccine. A nucleic acid encoding

25 the immunogenic peptide is incorporated into a genome or episome of the virus or bacteria. Optionally, the nucleic acid is incorporated in such a manner that the immunogenic peptide is expressed as a secreted protein or as a fusion protein with an outersurface protein of a virus or a transmembrane protein of a bacteria so that the peptide is displayed (Frenkel et al., 2000; 2001; WO 01/18169). Viruses or bacteria

30 used in such methods should be nonpathogenic or attenuated. Suitable viruses include bacteriophage such as filamentous bacteriophage, adenovirus, HSV, vaccinia and fowl pox. Fusion of an immunogenic peptide to HBsAg of HBV is particularly suitable.

The vaccine and/or therapeutic composition of the present invention may also comprise T-cells that bind to the N-terminal truncated and/or post-translationally modified A $\beta$  peptide of the invention. For example, T-cells can be activated against said peptides by expressing a human MHC class I gene and a human  $\beta$ -2-microglobulin gene from an insect cell line. An empty complex is formed on the surface of the cells that can be loaded with the N-terminal truncated and/or post-translational modified A $\beta$  peptide of the invention. T-cells contacted with the cell line become specifically activated against the peptides of the invention (US 5,314,813). Insect cell lines expressing an MHC class II antigen can similarly be used to activate CD4 T-cells.

In prophylactic applications, vaccine compositions are administered to a mammal susceptible to, or at risk of developing a disease associated with  $\beta$ -amyloid formation and/or aggregation in an amount sufficient to eliminate or reduce the risk or delay the onset of said disease. In therapeutic applications, compositions are administered to a mammal suspected of, or already suffering from such a disease in an amount sufficient to cure, or at least partially arrest, the symptoms of the disease and its complications. An amount adequate to accomplish this is defined as a therapeutically- or pharmaceutically-effective dose. In both prophylactic and therapeutic regimes, agents are usually administered in several dosages until a sufficient immune response has been achieved. Typically, the immune response is monitored and repeated dosages are given if the immune response starts to fade.

Effective doses of the vaccine and therapeutic compositions of the present invention, vary depending upon many different factors, including means of administration, target site, physiological state of the mammal, whether the patient is a human or an animal, other medications administered, and whether treatment is prophylactic or therapeutic. Treatment dosages need to be titrated to optimize safety and efficacy. The amount of immunogen depends on whether adjuvant is also administered, with higher dosages being required in the absence of adjuvant. The amount of an immunogen for administration sometimes varies from 1-500  $\mu$ g per mammal and more usually from 5-500  $\mu$ g per injection for human administration. Occasionally, a higher dose of 1-2 mg per injection is used. Typically about 10, 20, 50 or 100  $\mu$ g is used for each human injection. The timing of injections can vary significantly from once a day, to once a

year, to once a decade. On any given day that a dosage of immunogen is given, the dosage is greater than 1 µg/patient and usually greater than 10 µg/patient if adjuvant is also administered. In the absence of adjuvant, the dosage is greater than 10 µg/patient and usually greater than 100 µg/patient. A typical regimen consists of an

5 immunization followed by booster injections at 6 weekly intervals. Another regimen consists of an immunization followed by booster injections 1, 2 and 12 months later. Another regimen consists of an injection every two months for life. Alternatively, booster injections can be on an irregular basis as indicated by monitoring of immune response.

10 For passive immunization with an antibody, the dosage ranges from about 0.0001 to 100 mg/kg, and more usually 0.01 to 5 mg/kg of the host body weight. Doses for nucleic acids encoding immunogens range from about 10 ng to 1 g, 100 ng to 100 mg, 1 µg to 10 mg, or 30 to 300 µg DNA per patient. Doses for infectious viral vectors vary from 10 to  $10^9$ , or more virions per dose.

15 Agents for inducing an immune response can be administered by parenteral, topical, intravenous, oral, subcutaneous, intraperitoneal, intranasal or intramuscular means for prophylactic and/or therapeutic treatment. The most typical route of administration is subcutaneous, although others can be equally effective. The next most common is intramuscular injection. This type of injection is most typically performed in the arm

20 or leg muscles. Intravenous injections as well as intraperitoneal injections, intraarterial, intracranial, or intradermal injections are also effective in generating an immune response. In some methods, agents are injected directly into a particular tissue where deposits are accumulated. Intranasal immunization was successfully used to increase the production of anti-A $\beta$  antibodies in wildtype mice (Lemere et al.

25 2000b,c).

Vaccine or therapeutic compositions of the invention can optionally comprise other agents that are at least partly effective in treatment of diseases associated with  $\beta$ -amyloid formation and/or aggregation. In the case of Alzheimer's and Down's syndrome, in which  $\beta$ -amyloid aggregation occurs in the brain, the vaccine or therapeutic composition of the invention may also comprise other agents that increase passage of the active components of the composition of the invention across the blood-brain barrier. A peptide, protegrin PG-1, belonging to the family of beta-stranded antimicrobial peptides, for example, has been successfully used to deliver

therapeutic compounds into eucaryotic cells (Drin and Temsamani, 2002). Other strategies to enhance drug delivery across the blood brain barrier, especially vector mediated strategies have been reviewed by Temsamani et al. (2001).

Immunogenic agents of the invention, such as peptides, are sometimes administered in combination with an adjuvant. The term “adjuvant” refers to a compound that, when administered in conjunction with an antigen, augments the immune response to the antigen, but, when administered alone, does not generate an immune response to the antigen. Adjuvants can augment an immune response by several mechanisms including lymphocyte recruitment, stimulation of B and/or T cells, and stimulation of macrophages. A variety of adjuvants can be used in combination with the N-terminal truncated and/or post-translationally modified A $\beta$  peptides of the invention in order to elicit an immune response. Preferred adjuvants augment the intrinsic response to an immunogen without causing conformational changes in the immunogen that affect the qualitative form of the response. Preferred adjuvants include alum, 3 De-O-acylated monophosphoryl lipid A (MPL) (GB 2220211). QS21 is a triterpene glycoside or saponin isolated from the bark of the Quillaja Saponaria Molina tree found in South America (Kensil et al., 1995; US 5,057,540). Other adjuvants are oil in water emulsions (such as squalene or peanut oil), optionally in combination with immune stimulants, such as monophosphoryl lipid A (Stoute et al., 1997). Another adjuvant is CpG (Davis et al., 1998). Alternatively, the N-terminal truncated and/or post-translationally modified A $\beta$  peptide can be coupled to an adjuvant. For example, a lipopeptide version of the N-terminal truncated and/or post-translationally modified A $\beta$  peptide can be prepared by coupling palmitic acid or other lipids directly to the N-terminal truncated and/or post-translational modified A $\beta$  peptide as described for hepatitis D antigen vaccination (Livingston, 1997). However, such coupling should not substantially change the conformation of the N-terminal truncated and/or post-translational modified A $\beta$  peptide so as to affect the nature of the immune response thereto.

A preferred class of adjuvants is aluminum salts (alum), such as aluminum hydroxide, aluminum phosphate, aluminum sulfate. Such adjuvants can be used with or without other specific immunostimulating agents such as MPL or 3-DMP, QS21, polymeric or monomeric amino acids such as polyglutamic acid or polylysine. Another class of adjuvants is oil-in-water emulsion formulations. Such adjuvants can be used with or

without other specific immunostimulating agents such as muramyl peptides (e.g., N-acetylmuramyl-L-threonyl-D-isoglutamine (thr-MDP), N-acetyl-normuramyl-L-alanyl-D-isoglutamine (nor-MDP), N-acetylmuramyl-L-alanyl-D-isoglutaminyl-L-alanine-2-(1'-2'dipalmitoyl-sn-glycero-3-hydroxyphosphoryloxy)-ethylamine (MTP-PE), N-acetylglucosaminyl-N-acetylmuramyl-L-Al-D-isoglu-L-Ala-dipalmitoxy propylamide (DTP-DPP) theramide<sup>TM</sup>), or other bacterial cell wall components. Oil-in-water emulsions include (a) MF59 (WO 90/14837), containing 5% Squalene, 0.5% Tween 80, and 0.5% Span 85 (optionally containing various amounts of MTP-PE) formulated into submicron particles using a microfluidizer such as Model 110Y 5 microfluidizer (Microfluidics, Newton, MA, US), (b) SAF, containing 10% Squalane, 0.4% Tween 80, 5% pluronic-blocked polymer L121, and thr-MDP, either 10 microfluidized into a submicron emulsion or vortexed to generate a larger particle size emulsion, and (c) Ribi<sup>TM</sup> adjuvant system (RAS), (Ribi Immunochem, Hamilton, MT, US) containing 2% squalene, 0.2% Tween 80, and one or more bacterial cell wall 15 components from the group consisting of monophosphorylipid A (MPL), trehalose dimycolate (TDM), and cell wall skeleton (CWS), preferably MPL + CWS (Detox<sup>TM</sup>). Another class of preferred adjuvants is saponin adjuvants, such as Stimulon<sup>TM</sup> (QS21, Aquila, Worcester, MA) or particles generated therefrom such as ISCOMs (immunostimulating complexes) and ISCOMATRIX. Other adjuvants include 20 Complete Freund's Adjuvant (CFA) and Incomplete Freund's Adjuvant (IFA). Other adjuvants include cytokines, such as interleukins (IL-1, IL-2, and IL-12), macrophage colony stimulating factor (M-CSF), tumor necrosis factor (TNF). Published mucosal adjuvants include bacterial enterotoxins such as cholera toxin (CT) and E. coli LT, which are ~80% homologous (Dallas and Falkow, 1980).

An adjuvant can be administered with an immunogen as a single composition, or can be administered before, concurrent with or after administration of the immunogen. Immunogen and adjuvant can be packaged and supplied in the same vial or can be packaged in separate vials and mixed before use. Immunogen and adjuvant are typically packaged with a label indicating the intended therapeutic application. If 25 immunogen and adjuvant are packaged separately, the packaging typically includes instructions for mixing before use. The choice of an adjuvant and/or carrier depends on the stability of the vaccine containing the adjuvant, the route of administration, the dosing schedule, the efficacy of the adjuvant for the species being vaccinated, and, in 30

humans, a pharmaceutically acceptable adjuvant is one that has been approved or is approvable for human administration by pertinent regulatory bodies. For example, Complete Freund's adjuvant is not suitable for human administration. Alum, MPL and QS21 are preferred. Optionally, two or more different adjuvants can be used

5 simultaneously. Preferred combinations include alum with MPL, alum with QS21, MPL with QS21, and alum, QS21 and MPL together. Also, Incomplete Freund's adjuvant can be used (Jensen et al., 1998), optionally in combination with any of alum, QS21, and MPL and all combinations thereof.

The preferred form of the vaccine and/or therapeutic composition depends on the

10 intended mode of administration and application. The compositions can also include, depending on the formulation desired, pharmaceutically-acceptable, non-toxic carriers or diluents, which are defined as vehicles commonly used to formulate pharmaceutical compositions for animal or human administration. The diluent is selected so as not to affect the biological activity of the combination. Examples of

15 such diluents are distilled water, physiological phosphate-buffered saline, Ringer's solutions, dextrose solution, and Hank's solution. In addition, the pharmaceutical composition or formulation may also include other carriers, adjuvants, or nontoxic, nontherapeutic, nonimmunogenic stabilizers and the like.

The vaccine or pharmaceutical compositions can also include large, slowly

20 metabolized macromolecules such as proteins, polysaccharides, polylactic acids, polyglycolic acids and copolymers (such as latex functionalized sepharose, agarose, cellulose, and the like), polymeric amino acids, amino acid copolymers, and lipid aggregates (such as oil droplets or liposomes). Additionally, these carriers can function as immunostimulating agents (i.e., adjuvants).

25 For parenteral administration, the compositions of the invention can be administered as injectable dosages of a solution or suspension of the substance in a physiologically acceptable diluent with a pharmaceutical carrier that can be a sterile liquid such as water oils, saline, glycerol, or ethanol. Also encapsulation into biodegradable microparticles can be used as a parenteral delivery system (Brayden et al., 2001).

30 Additionally, auxiliary substances, such as wetting or emulsifying agents, surfactants, pH buffering substances and the like can be present in the vaccine and/or therapeutic compositions. Other components of pharmaceutical compositions are those of petroleum, animal, vegetable, or synthetic origin, for example, peanut oil, soybean oil,

and mineral oil. In general, glycols such as propylene glycol or polyethylene glycol are preferred liquid carriers, particularly for injectable solutions.

Typically, compositions are prepared as injectables, either as liquid solutions or suspensions. Solid forms suitable for solution in, or suspension in, liquid vehicles

5 prior to injection can also be prepared. The preparation also can be emulsified or encapsulated in liposomes or micro particles such as polylactide, polyglycolide, or copolymer for enhanced adjuvant effect, as discussed above (Langer, 1990; Langer, 1997). The compositions of this invention can be administered in the form of a depot injection or implant preparation which can be formulated in such a manner as to  
10 permit a sustained or pulsatile release of the active ingredient.

Additional formulations suitable for other modes of administration include oral, intranasal, and pulmonary formulations, suppositories, and transdermal applications.

For suppositories, binders and carriers include, for example, polyalkylene glycols or triglycerides. Such suppositories can be formed from mixtures containing the active

15 ingredient in the range of 0.5% to 10%, preferably 1% to 2%. Oral formulations include excipients, such as pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, and magnesium carbonate. These compositions take the form of solutions, suspensions, tablets, pills, capsules, sustained release formulations or powders and contain 10% to 95% of active ingredient,  
20 preferably 25% to 70%.

Topical application can result in transdermal or intradermal delivery. Topical administration can be facilitated by co-administration of the agent with cholera toxin or detoxified derivatives or subunits thereof or other similar bacterial toxins (Glenn et al., 1998). Co-administration can be achieved by using the components as a mixture  
25 or as linked molecules obtained by chemical crosslinking or expression as a fusion protein.

Alternatively, transdermal delivery can be achieved using a skin path or using transferosomes (Paul et al., 1995; Cevc et al., 1998).

Further techniques for formulation and administration of drugs can also be found in

30 "Remington's Pharmaceutical Sciences".

The vaccine and therapeutic compositions of the present invention can thus be used for the prevention and/or treatment of any disease associated with  $\beta$ -amyloid

formation and/or aggregation. In a preferred embodiment the vaccine and therapeutic compositions of the invention are used for the prevention and/or treatment of Alzheimer's disease or Down's syndrome.

The invention thus relates to a N-terminal truncated and/or post-translationally modified A $\beta$  peptide as described above, an antibody or T-cell specific for a N-terminal truncated and/or post-translationally modified A $\beta$  peptide as described above, or a nucleic acid encoding a N-terminal truncated and/or post-translationally modified A $\beta$  peptide as described above for use as a prophylactic vaccine for the prevention of a disease associated with  $\beta$ -amyloid formation and/or aggregation. The invention thus also relates to the use of a N-terminal truncated and/or post-translationally modified A $\beta$  peptide as discussed above, an antibody or T-cell specific for a N-terminal truncated and/or post-translationally modified A $\beta$  peptide as discussed above, or a nucleic acid encoding a N-terminal truncated and/or post-translationally modified A $\beta$  peptide as discussed above for the manufacture of a prophylactic vaccine for the prevention of a disease associated with  $\beta$ -amyloid formation and/or aggregation.

In non-demented humans, prophylaxis can begin at any age (e.g., 10, 20, 30). Usually, however, it is not necessary to begin prophylaxis until a patient reaches 40, 50, 60 or 70. Prophylaxis typically encompasses multiple dosages over a period of time. The response can be monitored by assaying antibody, or activated T-cell or B-cell responses to the N-terminal truncated and/or post-translationally modified peptide over time (see further). If the response falls, a booster dosage is indicated.

The invention further relates to a N-terminal truncated and/or post-translationally modified A $\beta$  peptide as discussed above, an antibody specific for a N-terminal truncated and/or post-translationally modified A $\beta$  peptide as discussed above, or a nucleic acid encoding a N-terminal truncated and/or post-translationally modified A $\beta$  peptide as discussed above for use as a therapeutic for the treatment of a disease associated with  $\beta$ -amyloid formation and/or aggregation. The invention thus also relates to the use of a N-terminal truncated and/or post-translationally modified A $\beta$  peptide as discussed above, an antibody specific for a N-terminal truncated and/or post-translationally modified A $\beta$  peptide as discussed above, or a nucleic acid encoding a N-terminal truncated and/or post-translationally modified A $\beta$  peptide as

discussed above for the manufacture of a therapeutic for the treatment of a disease associated with  $\beta$ -amyloid formation and/or aggregation.

In accordance, the present invention also encompasses a method for the prevention and/or treatment, in an mammal, of a disease associated with  $\beta$ -amyloid formation

- 5 and/or, said method comprising the administration, to said mammal, of a vaccine composition or a therapeutic composition as described above.

#### Diagnostic and theranostic kits

Another aspect of the present invention relates to a diagnostic or theranostic kit comprising a preparation of N-terminal truncated and/or post-translationally modified peptide as described above, comprising a preparation of N-terminal APP soluble fragment of the invention or C-terminal fragment thereof as described above, or comprising an antibody specifically recognizing said peptide or fragments.

In one embodiment, the kit thus contains a peptide or peptide fragment that specifically binds to antibodies recognizing N-terminal truncated and/or post-translationally modified A $\beta$  peptide or recognizing N-terminal APP soluble of the invention (or C-terminal fragment thereof), or that reacts with T-cells specific for N-terminal truncated and/or post-translationally modified A $\beta$  peptide or specific for N-terminal APP soluble fragment of the invention (or C-terminal fragment thereof). The

20 kit may typically also include a label (see below). For the detection of antibodies to N-terminal truncated and/or post-translationally modified A $\beta$  peptide or N-terminal APP soluble fragment of the invention (or C-terminal fragment thereof), the label is typically in the form of labelled anti-Ig antibodies. For detection of antibodies, the N-terminal truncated and/or post-translationally modified A $\beta$  peptide or N-terminal APP 25 soluble fragment of the invention or C-terminal fragment thereof can be supplied prebound to a solid phase, such as to the wells of a microtiter dish. For detection of reactive T- cells, the label can be supplied as 3H-thymidine to measure a proliferative response.

A diagnostic or theranostic kit comprising N-terminal truncated and/or post-translationally modified A $\beta$  peptide will aid in methods of detecting an immune response against N-terminal truncated and/or post-translationally modified A $\beta$  peptide in a mammal. The immune response can be determined from the presence of antibodies or T- cells that specifically bind to the N-terminal truncated and/or post-

translationally modified A<sub>β</sub> peptide. The methods are particularly useful for monitoring a course of treatment being administered to a mammal. The kit can be used to monitor both therapeutic treatment on symptomatic patients and prophylactic treatment on asymptomatic patients. In accordance, the present invention relates to a  
5 preparation comprising N-terminal truncated and/or post-translationally modified A<sub>β</sub> peptide for use as a diagnostic or theranostic for the measurement of the immune response induced in a mammal by vaccination or therapeutic application with respectively a vaccine composition or a therapeutic composition of the invention. The invention thus also relates to the use of a preparation comprising N-terminal truncated  
10 and/or post-translationally modified A<sub>β</sub> peptide for the manufacture of a diagnostic or theranostic kit for the measurement of the immune response induced in a mammal by vaccination or therapeutic application with respectively a vaccine composition or a therapeutic composition of the invention.

The diagnostic or theranostic kit of the invention thus will aid in a method for the  
15 measurement, in a mammal, of the immune response induced by vaccination or therapeutic application with respectively a vaccine composition or a therapeutic composition of the invention. Said method comprises the following steps:

- (a) Determining, in a sample obtained from said mammal, the amount of antibody or reactive T-cell specific for a N-terminal truncated and/or post-translationally modified peptide;  
20
- (b) Comparing the amount determined in step (a) with the amount of antibody or reactive T-cell specific for said N-terminal truncated and/or post-translationally modified peptide present in the mammal before vaccination or therapeutic application with the vaccine or therapeutic composition of the  
25 invention;
- (c) Concluding, from the comparison in step (b), whether the mammal is responding to the vaccination or therapy, an increased amount of antibody or reactivated T-cell specific for said N-terminal truncated and/or post-translationally modified peptide being an indication that the mammal is responding to the vaccination or therapy.  
30

The antibody specific for said N-terminal truncated and/or post-translationally modified peptide can be detected by an immunoassay. As used herein, an “immunoassay” is an assay that utilizes an antibody to specifically bind to the antigen

- (i.e. the N-terminal truncated and/or post-translationally modified peptide). The immunoassay is thus characterized by detection of specific binding of proteins to antibodies. Immunological methods include but are not limited to fluid or gel precipitation reactions, immunodiffusion (single or double), agglutination assays,  
5 immunoelectrophoresis, radioimmunoassays (RIA), enzyme-linked immunosorbent assays (ELISA), Western blots, liposome immunoassays (LIA; Monroe et al., 1986), complement-fixation assays, immunoradiometric assays, fluorescent immunoassays, protein A immunoassays or immunoPCR. An overview of different immunoassays is given in Wild D. (2001) and Ghindilis et al. (2002).
- 10 T-cells that recognize a particular epitope can be identified by in vitro assays that measure antigen-dependent proliferation, as determined by <sup>3</sup>H-thymidine incorporation by primed T cells in response to an epitope (Burke et al., 1994), by antigen-dependent killing (cytotoxic T lymphocyte assay; Tigges et al. 1996) or by cytokine secretion. The presence of a cell-mediated immunological response can be  
15 determined by proliferation assays (CD4+ T cells) or CTL (cytotoxic T lymphocyte) assays (Burke et al. 1994; Tigges et al., 1996).

The method entails determining a baseline value of an immune response in a patient before administering a dosage of the vaccine or therapeutic composition, and  
20 comparing this with a value for the immune response after vaccination or therapy. A significant increase (i.e., greater than is the typical margin of experimental error in repeat measurements of the same sample, expressed as one standard deviation from the mean of such measurements) in value of the immune response signals a positive vaccination or therapy outcome (i.e., that administration of the vaccine or therapeutic  
25 composition has achieved or augmented an immune response). If the value for immune response does not change significantly, or decreases, a negative vaccination or therapy outcome is indicated. In general, patients undergoing an initial course of treatment with a vaccine or therapeutic composition are expected to show an increase in immune response with successive dosages, which eventually reaches a plateau.  
30 Administration of the vaccine or therapeutic composition is generally continued while the immune response is increasing. Attainment of the plateau is an indicator that the administration can be discontinued or reduced in dosage or frequency.

The term “sample” refers to any source of biological material, for instance body fluids, brain extract, peripheral blood, mucus or any other sample comprising the antibodies or

the reactive T-cells to the N-terminal truncated and/or post-translationally modified peptide. In a preferred embodiment, the level of said antibodies or reactive T-cells is determined in a body fluid sample of the mammal. The term "body fluid" refers to all fluids that are present in the mammalian body including but not limited to blood,

5 lymph, urine and cerebrospinal fluid (CSF) comprising the antibodies or reactive T-cells to be detected. The term "cerebrospinal fluid" or "CSF" is intended to include whole cerebrospinal fluid or derivatives of fractions thereof well known to those skilled in the art. Thus, a cerebrospinal fluid sample can include various fractionated forms of cerebrospinal fluid or can include various diluents added to facilitate storage  
10 or processing in a particular assay. Such diluents are well known to those skilled in the art and include various buffers, preservatives and the like. In another preferred embodiment, the level of antibody is detected in a blood sample of the mammal. The blood sample may include a plasma sample or a serum sample.

15 In another embodiment of the invention, the diagnostic or theranostic kit of the invention comprises a peptide or peptide fragment that specifically binds to antibodies recognizing N-terminal truncated and/or post-translationally modified A $\beta$  variant or to N-terminal APP soluble fragment of the invention or that reacts with T-cells specific for N-terminal truncated and/or post-translationally modified A $\beta$  variant or for N-  
20 terminal APP soluble fragment of the invention. A diagnostic or theranostic kit comprising N-terminal truncated and/or post-translationally modified A $\beta$  peptide, N-terminal APP soluble fragment of the invention or C-terminal fragment thereof will aid in methods of detecting mammals susceptible to or at risk of developing a disease associated with  $\beta$ -amyloid formation and/or aggregation. The susceptibility to or the  
25 risk of developing such a disease can be determined from the presence of antibodies or T- cells that specifically bind to the N-terminal truncated and/or post-translationally modified A $\beta$  peptide, to the N-terminal APP soluble fragment of the invention or to the C-terminal fragment thereof. In accordance, the present invention relates to a preparation comprising a N-terminal truncated and/or post-translationally modified  
30 A $\beta$  peptide, a N-terminal APP soluble fragment of the invention, or a C-terminal fragment thereof for use as a diagnostic or theranostic for determining, in a mammal, the susceptibility to a disease associated with  $\beta$ -amyloid formation and/or aggregation or for determining, in a mammal, the risk of developing a disease associated with  $\beta$ -

amyloid formation and/or aggregation. The invention thus also relates to the use of a preparation comprising a N-terminal truncated and/or post-translationally modified A $\beta$  peptide, a N-terminal APP soluble fragment of the invention or a C-terminal fragment thereof for the manufacture of a diagnostic or theranostic kit for  
5 determining, in a mammal, the susceptibility to a disease associated with  $\beta$ -amyloid formation and/or aggregation or for determining, in a mammal, the risk of developing a disease associated with  $\beta$ -amyloid formation and/or aggregation.

The diagnostic or theranostic kit of the invention thus will aid in a method for the measurement, in a mammal, the susceptibility to a disease associated with  $\beta$ -amyloid  
10 formation and/or aggregation or the risk of developing a disease associated with  $\beta$ -amyloid formation and/or aggregation. Said method comprises the following steps:

(a) Determining, in a sample obtained from said mammal, the amount of antibody or reactive T-cells specific for a N-terminal truncated and/or post-translationally modified A $\beta$  peptide, for a N-terminal APP soluble fragment of  
15 the invention or for a C-terminal fragment thereof;

(b) Comparing the amount determined in step (a) with the amount of said antibody or reactive T-cells in a control mammal;

(c) Concluding, from the comparison in step (b), whether the mammal is  
susceptibility to a disease associated with  $\beta$ -amyloid formation and/or aggregation or whether the mammal is at risk of developing a disease associated with  $\beta$ -amyloid formation and/or aggregation, an increased amount  
20 of antibody or reactivated T-cells specific for said N-terminal truncated and/or post-translationally modified A $\beta$  peptide, for said N-terminal APP soluble fragment of the invention or for said C-terminal fragment thereof being an indication that the mammal is susceptible to or at risk of developing a disease associated with A $\beta$  formation and/or aggregation.  
25

In another embodiment, the diagnostic or theranostic kit of the invention comprises an antibody that specifically recognizes N-terminal truncated and/or post-translationally modified A $\beta$  peptide or N-terminal APP soluble fragment of the invention or C-terminal fragment thereof. This kit can then be used to determine, in a mammal, the level of N-terminal truncated and/or post-translationally modified A $\beta$  variant or N-terminal APP soluble fragment of the invention, indicating if the mammal is  
30

susceptible to a disease associated with  $\beta$ -amyloid formation and/or aggregation, if the mammal is at risk of developing a disease associated with  $\beta$ -amyloid formation and/or aggregation, if  $\beta$ -amyloid deposits have been cleared, or the level of  $\beta$ -amyloid burden in the mammal. In accordance, the present invention relates to an antibody that

5 specifically recognizes N-terminal truncated and/or post-translationally modified A $\beta$  variant or N-terminal APP soluble fragment of the invention for use as a diagnostic or theranostic for determining, in a mammal, susceptibility to a disease associated with  $\beta$ -amyloid formation and/or aggregation, for determining, in a mammal, the risk of developing a disease associated with  $\beta$ -amyloid formation and/or aggregation, for

10 screening of the clearance of  $\beta$ -amyloid deposition in a mammal, or for predicting the level of  $\beta$ -amyloid burden in a mammal. The invention further relates to the use of an antibody that specifically recognizes N-terminal truncated and/or post-translationally modified A $\beta$  variant or N-terminal APP soluble fragment of the invention for the manufacture of diagnostic or theranostic kit for determining, in a mammal,

15 susceptibility to a disease associated with  $\beta$ -amyloid formation and/or aggregation, for determining, in a mammal, the risk of developing a disease associated with  $\beta$ -amyloid formation and/or aggregation, for screening of the clearance of  $\beta$ -amyloid deposition in a mammal, or for predicting the level of  $\beta$ -amyloid burden in a mammal.

A preferred kit for carrying out the method of the invention comprises:

- 20 - an antibody (primary antibody) which forms an immunological complex with the N-terminal truncated and/or post-translationally modified A $\beta$  variant or the N-terminal APP soluble fragment of the invention to be detected;
- 25 - a monoclonal antibody (secondary antibody) which specifically recognizes the N-terminal truncated and/or post-translationally modified A $\beta$  variant or the N-terminal APP soluble fragment of the invention to be detected;
- a marker either for specific tagging or coupling with said secondary antibody;
- appropriate buffer solutions for carrying out the immunological reaction between the primary antibody and the N-terminal truncated and/or post-translationally modified A $\beta$  variant or the N-terminal APP soluble fragment, between the secondary antibody and the primary antibody-N-
- 30

- terminal truncated and/or post-translationally modified A $\beta$  variant or -N-terminal APP soluble fragment complex and/or between the bound secondary antibody and the marker;
- possibly, for standardization purposes, a purified N-terminal truncated and/or post-translationally modified A $\beta$  peptide or a purified N-terminal APP soluble fragment (or a C-terminal fragment thereof).

The kit of the invention can be used in a method for determining, in a mammal, the susceptibility to a disease associated with  $\beta$ -amyloid formation and/or aggregation, for determining, in a mammal, the risk of developing a disease associated with  $\beta$ -amyloid formation and/or aggregation, for screening of the clearance of  $\beta$ -amyloid deposition in a mammal, or for predicting the level of  $\beta$ -amyloid burden in a mammal. Said method comprises the following steps:

- (a) Determining, in said mammal, the amount of N-terminal truncated and/or post-translationally modified A $\beta$  variant or the amount of N-terminal APP soluble fragment of the invention;
- (b) Comparing the amount determined in step (a) with the amount of N-terminal truncated and/or post-translationally modified A $\beta$  variant or N-terminal APP soluble fragment of the invention in a control mammal;
- (c) Concluding, from the comparison in step (b), whether the mammal is susceptible to a disease associated with  $\beta$ -amyloid formation and/or aggregation, whether the mammal is at risk of developing a disease associated with  $\beta$ -amyloid formation and/or aggregation, whether the  $\beta$ -amyloid deposition in the mammal is cleared, or what the level of  $\beta$ -amyloid burden is in said mammal.

An increase in the level of N-terminal truncated and/or post-translationally modified A $\beta$  variant in the brain of the tested mammal, for example, could be an indication of the mammal being susceptible to or at risk of developing a disease associated with  $\beta$ -amyloid formation and/or aggregation. It could also indicate that the A $\beta$  deposition in the mammal is not yet cleared. Increased levels of N-terminal truncated and/or post-translationally modified A $\beta$  variant in certain body fluids after vaccination or therapy, are an indication of the level of A $\beta$  burden (DeMattos et al., 2002). N-terminal APP soluble fragment will mainly be found in certain body fluids. The presence of these N-

terminal APP soluble fragments indicates an aberrant cleavage of APP, resulting in the formation of N-terminal truncated A $\beta$  variants and, consequently, in an increased susceptibility to or risk of developing a disease associated with  $\beta$ -amyloid formation and/or aggregation by the mammal.

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In an embodiment of the invention, the level of N-terminal truncated and/or post-translationally modified A $\beta$  variant or N-terminal APP soluble fragment of the invention can be determined by *in vivo* imaging. The level of N-terminal truncated and/or post-translationally modified A $\beta$  variant or N-terminal APP soluble fragment of the invention can be determined *in situ* by non-invasive methods including but not limited to brain imaging methods described by Arbit et al. (1995), Tamada et al. (1995), Wakabayashi et al. (1995), Huang et al. (1996), Sandrock et al. (1996), Mariani et al. (1997). These *in vivo* imaging methods may allow the localization and quantification of the N-terminal truncated and/or post-translationally modified A $\beta$  variant or N-terminal APP soluble fragment of the invention, for example, by use of labeled antibodies (see below) specifically recognizing said N-terminal truncated and/or post-translationally modified A $\beta$  variant or N-terminal APP soluble fragment of the invention. *In vivo* multiphoton microscopy (Bacska et al., 2001) can be used to image the presence of N-terminal truncated and/or post-translationally modified A $\beta$  variant or N-terminal APP soluble fragment of the invention using labeled antibodies specific for the N-terminal truncated and/or post-translationally modified A $\beta$  variant or the N-terminal APP soluble fragment of the invention.

In another embodiment of the invention, the level of N-terminal truncated and/or post-translationally modified A $\beta$  variant or N-terminal APP soluble fragment of the invention can be determined *in vitro*, in a sample obtained from the mammal. In accordance, the present invention also relates to a method for determining, in a mammal, the susceptibility to a disease associated with  $\beta$ -amyloid formation and/or aggregation, for determining, in a mammal, the risk of developing a disease associated with  $\beta$ -amyloid formation and/or aggregation, for screening of the clearance of  $\beta$ -amyloid deposition in a mammal, or for predicting the level of  $\beta$ -amyloid burden in a mammal, further characterized in that the amount of N-terminal truncated and/or post-translationally modified A $\beta$  variant or the amount of N-terminal APP soluble

fragment of the invention is determined on a sample obtained from said mammal. The invention thus comprises the following steps:

- (a) Determining, in a sample obtained from said mammal, the amount N-terminal truncated and/or post-translationally modified A $\beta$  variant or N-terminal APP soluble fragment of the invention;
- 5 (b) Comparing the amount determined in step (a) with the amount of N-terminal truncated and/or post-translationally modified A $\beta$  variant or N-terminal APP soluble fragment of the invention in a tissue sample obtained from a control mammal;
- 10 (c) Concluding, from the comparison in step (b), whether the mammal is susceptible to a disease associated with  $\beta$ -amyloid formation and/or aggregation, whether the mammal is at risk of developing a disease associated with  $\beta$ -amyloid formation and/or aggregation, whether the  $\beta$ -amyloid deposition in the mammal is cleared, or what the level of  $\beta$ -amyloid burden is
- 15 in said mammal.

The present invention further relates to a method for predicting the level of  $\beta$ -amyloid burden in a mammal (DeMattos et al., 2002), said method comprising the following steps:

- (a) Administration, to said mammal, of a vaccine composition or a therapeutic composition as described above;
  - 20 (b) Determining, in a biological fluid sample obtained from said mammal, the amount of N-terminal truncated and/or post-translationally modified  $\beta$ -amyloid variant;
  - (c) Comparing the amount determined in step (b) with the amount of N-terminal truncated and/or post-translationally modified  $\beta$ -amyloid variant in a biological fluid sample obtained from a control mammal;
  - 25 (d) Concluding, from the comparison in step (c), what the level of  $\beta$ -amyloid burden is in said mammal.
- 30 The level of N-terminal truncated and/or post-translationally modified  $\beta$ -amyloid variant or N-terminal APP soluble fragment of the invention can be detected by an immunoassay as discussed above. Immunoassays for detecting the N-terminal truncated and/or post-translationally modified A $\beta$  variant or N-terminal APP soluble

fragment of the invention may be either competitive or noncompetitive. Noncompetitive immunoassays are assays in which the amount of captured analyte (i.e. the N-terminal truncated and/or post-translationally modified A $\beta$  variant or N-terminal APP soluble fragment of the invention) is directly measured. In competitive assays, the amount of analyte (i.e. the N-terminal truncated and/or post-translationally modified A $\beta$  variant or N-terminal APP soluble fragment of the invention) present in the sample is measured indirectly by measuring the amount of an added (exogenous) analyte displaced (or competed away) from a capture agent (i.e. the antibody) by the analyte present in the sample. In one competition assay, a known amount of (exogenous) N-terminal truncated and/or post-translationally modified A $\beta$  peptide or N-terminal APP soluble fragment of the invention (or a C-terminal fragment thereof) is added to the sample and the sample is then contacted with the antibody. The amount of added (exogenous) N-terminal truncated and/or post-translationally modified A $\beta$  peptide or N-terminal APP soluble fragment of the invention (or C-terminal fragment thereof) bound to the antibody is inversely proportional to the concentration of the N-terminal truncated and/or post-translationally modified A $\beta$  variant or N-terminal APP soluble fragment of the invention in the sample before the exogenous N-terminal truncated and/or post-translationally modified A $\beta$  peptide or N-terminal APP soluble fragment of the invention (or C-terminal fragment thereof) is added.

In a preferred embodiment, the level of the N-terminal truncated and/or post-translationally modified A $\beta$  variant or N-terminal APP soluble fragment of the invention is determined by an immunoassay comprising at least the following steps:

(a) Contacting the N-terminal truncated and/or post-translationally modified A $\beta$  variant or N-terminal APP soluble fragment of the invention present in the sample with an antibody that specifically recognizes the N-terminal truncated and/or post-translationally modified A $\beta$  variant or the N-terminal APP soluble fragment of the invention, under conditions suitable for producing an antigen-antibody complex; and

(b) Detecting the immunological binding that has occurred between the antibody and the N-terminal truncated and/or post-translationally modified A $\beta$  variant or the N-terminal APP soluble fragment of the invention.

- In one preferred “sandwich” assay, for example, the antibodies can be bound directly to a solid substrate where they are immobilized. These immobilized antibodies then capture the N-terminal truncated and/or post-translationally modified A $\beta$  variant or N-terminal APP soluble fragment of the invention present in the sample which are subsequently detected with a second antibody. In a preferred embodiment, the N-terminal truncated and/or post-translationally modified A $\beta$  variant or the N-terminal APP soluble fragment of the invention can be detected by a sandwich ELISA comprising the following steps:
- 5        10        15        20        25        30
- (a) Bringing said N-terminal truncated and/or post-translationally modified A $\beta$  variant or said N-terminal APP soluble fragment of the invention present in the sample, into contact with an antibody (primary antibody or capturing antibody) recognizing said N-terminal truncated and/or post-translationally modified A $\beta$  variant or said N-terminal APP soluble fragment of the invention, under conditions being suitable for producing an antigen-antibody complex;
  - (b) Bringing the complex formed between said N-terminal truncated and/or post-translationally modified A $\beta$  variant or said N-terminal APP soluble fragment of the invention and said primary antibody into contact with another antibody (secondary antibody or detector antibody) specifically recognizing the N-terminal truncated and/or post-translationally modified A $\beta$  variant or the N-terminal APP soluble fragment of the invention, under conditions being suitable for producing an antigen-antibody complex;
  - (c) Bringing the antigen-antibody complex into contact with a marker either for specific tagging or coupling with said secondary antibody, with said marker being any possible marker known to the person skilled in the art;
  - (d) Possibly also, for standardization purposes, bringing the antibodies in contact with a purified N-terminal truncated and/or post-translationally modified A $\beta$  peptide or N-terminal APP soluble fragment of the invention (or a C-terminal fragment thereof) reactive with both antibodies.
- Advantageously, the secondary antibody itself carries a marker or a group for direct or indirect coupling with a marker.

The antibodies used in the diagnostic or theranostic methods of the present invention may be labeled by an appropriate label. The particular label or detectable group used in the assay is not a critical aspect of the invention, so long as it does not significantly interfere with the specific binding of the antibody used in the assay. The detectable group can be any material having a detectable physical or chemical property. Such detectable labels have been well developed in the field of immunoassays and, in general, almost any label useful in such methods can be applied to the method of the present invention. Thus, a label is any composition detectable by spectroscopic, photochemical, biochemical, immunochemical, electrical, optical or chemical means.

Useful labels in the present invention include but are not limited to magnetic beads (e.g. Dynabeads<sup>TM</sup>), fluorescent dyes (e.g. fluorescein isothiocyanate, texas red, rhodamine), radiolabels (e.g. <sup>3</sup>H, <sup>125</sup>I, <sup>35</sup>S, <sup>14</sup>C, or <sup>32</sup>P), enzymes (e.g. horse radish peroxidase, alkaline phosphatase and others commonly used in an ELISA), and colorimetric labels such as colloidal gold, colored glass or plastic (e.g. polystyrene, polypropylene, latex, etc.) beads.

The label may be coupled directly or indirectly to the desired component of the assay according to methods well known in the art. As indicated above, a wide variety of labels may be used, with the choice of label depending on the sensitivity required, the ease of conjugation with the compound, stability requirements, the available instrumentation and disposal provisions. Non-radioactive labels are often attached by indirect means. Generally, a ligand molecule (e.g. biotin) is covalently bound to the antibody. The ligand then binds to an anti-ligand (e.g. streptavidin) molecule, which is either inherently detectable or covalently bound to a signal system, such as a detectable enzyme, a fluorescent compound, or a chemiluminescent compound. A number of ligands and anti-ligands can be used. Where a ligand has a natural anti-ligand, for example, biotin, thyroxine, and cortisol, it can be used in conjunction with the labeled, naturally occurring anti-ligands. Alternatively, a haptenic or antigenic compound can be used in combination with an antibody. The antibodies can also be conjugated directly to signal generating compounds, for example., by conjugation with an enzyme or fluorophore. Enzymes of interest as labels will primarily be hydrolases, particularly phosphatases, esterases and glycosidases, or oxidoreductases, particularly peroxidases. Fluorescent compounds include fluorescein and its derivatives, rhodamine and its derivatives, dansyl, umberliferone, etc. Chemiluminescent compounds include luciferin, and 2,3-dihydrophthiazinediones,

for example, luminol. A review of other labeling or signal producing system is available in US patent No. 4,391,904.

Means for detecting labels are well known in the art. Thus, for example, where the label is a radioactive label, means for detection include a scintillation counter or photographic film as in autoradiography. Where the label is a fluorescent label, it may be detected by exciting the fluorophore with the appropriate wavelength of light and detecting the resulting fluorescence. The fluorescence may be detected visually, by means of a photographic film, by the use of electronic detectors such as charge coupled devices (CCDs) or photomultipliers and the like. Similarly, enzyme labels may be detected by providing the appropriate substrates for the enzyme and detecting the resulting reaction product. Finally simple colorimetric labels may be detected simply by observing the color associated with the label.

Some assay formats do not require the use of labeled components. For instance, agglutination assays can be used to detect the presence of the target antibodies. In this case, antigen-coated particles are agglutinated by samples comprising the target antibodies. In this format, none of the components need be labeled and the presence of the target antibody is detected by simple visual inspection.

Throughout this specification and the claims which follow, unless the context requires otherwise, the word "comprise", and variations such as "comprises" and "comprising", will be understood to imply the inclusion of a stated integer or step or group of stated integers or steps but not to the exclusion of any other integer or step or group of integers or steps.

## **EXAMPLES**

### **Example 1: Characterization of the A $\beta$ peptides in fully developed Alzheimer's disease patients and in non-demented patients**

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#### **1. Material and methods**

##### Patients

All of the brain autopsy materials used in the present study were from the brain bank

10 kept at INSERM (Lille, France). Five AD cases and five non-demented cases have already been described (Delacourte et al., 2002; Delacourte et al., 1999). The five AD cases fulfilled the neuropathological diagnostic criteria of AD as established by the National Institute on Aging and the Reagan Institute Working Group on diagnostic criteria for the neuropathological assessment of Alzheimer disease (Hyman and 15 Trojanowski, 1997). The five non-demented cases correspond to neurofibrillary stages I and II and Tau pathology stages 1 to 6 according to Delacourte et al. (1999; 2002) and stage B for amyloid deposition, according to neuropathological staging of Braak and Braak (1991). At autopsy, one brain hemisphere was deep-frozen and used for biochemical analysis and the other hemisphere was formalin-fixed for both 20 neuropathological examination and histochemistry.

##### Antibodies

The amino-terminal regions of A $\beta$  peptides were analysed with WO2 (Abeta, GmBH, 25 Germany) and 6E10 (Senetek, MO, USA) antibodies. These recognise the amino-acid sequences 5-8 and 4-13 respectively, of A $\beta$ . A $\beta$  x-42 species were studied using 21F12 antibody and ADA42 antiserum. A $\beta$  x-40 species were analysed with our antiserum ADA40 (Delacourte et al., 2002).

##### Formic acid isolation of A $\beta$ aggregates and two-dimensional gel electrophoresis

30 The brain tissue samples from the temporal, frontal, parietal and occipital cortex were processed as already described (Delacourte et al., 2002). Formic acid (Prolabo, Fontenay s/Bois, France) extracted brain tissue homogenate (100  $\mu$ L) was evaporated under nitrogen and dissolved in 400  $\mu$ l of two-dimensional electrophoresis lysis buffer

(7 M urea, 2 M thiourea, 4% Triton X-100, 20 mM DTT and 0.6% Pharmalytes™ pH 3-10). The sample was sonicated and an IPG strip pH 4-7 (BioRad, Marnes la Coquette, France) was equilibrated with the sample for 15 hrs (Sergeant et al., 2002). Isoelectrofocusing was performed using the Protean IEF cell following the manufacturer's (BioRad, Marnes la Coquette, France) instructions. Polypeptides were resolved on Tris-Tricine gels as described earlier (Sergeant et al., 2002). The gels were transferred for immunodetection using the Multiphor transfer unit (Amersham-Pharmacia Biotech, Saclay, France), according to the manufacturer's instructions, or they were stained with Coomassie Brilliant Blue G250 (Sigma, France) for mass spectrometric analyses. Isoelectric points, molecular weights and the amount of each A $\beta$  peptide variant were determined using Melanie III 2D gel analysis software (Genebio, Geneva, Switzerland).

#### Mass spectrometry characterization

Coomassie Blue stained polypeptides spots were cut into 1 mm<sup>2</sup> gel pieces and washed twice with 50% CH<sub>3</sub>CN in 25 mM Tris-HCl pH 9. Gel pieces were dehydrated in a Speed-Vac and then in-gel digested overnight with 10 ng of Endoproteinase Lys-C (EC 3.4.21.19, Roche Molecular Biochemicals, Meylan, France) in 3  $\mu$ L of Tris-HCl pH 9. The resulting digested peptides were recovered in 10  $\mu$ L of 50% CH<sub>3</sub>CN and 1% trifluoroacetic acid (TFA). Samples were then prepared by the dry-droplet method. One mL of the peptide mixture was mixed with freshly dissolved  $\alpha$ -Cyano-4-hydroxycinnamic acid 0.5 mL (5 mg/mL in 50% CH<sub>3</sub>CN and 0.1% TFA), and spotted on the sample plate. The dry spot was then washed with 5  $\mu$ L of 0.1%TFA. Mass spectrometry was performed with a MALDI-TOF Voyager-DE-STR (Applied Biosystems, Palo Alto, CA) set to the following parameters: positive mode, reflector, voltage 20 kV, grid 61%, delayed extraction 90 ns, low mass gate 500 amu. The laser energy required to desorb/ionise samples was kept to a low value, compatible with a good signal/noise ratio. Spectra were calibrated externally using the [M+H<sup>+</sup>] monoisotopic ions from trypsinized lysozyme.

## **2. Characterization of the A $\beta$ peptides in brain obtained from fully developed Alzheimer's disease patients**

We first characterized the A $\beta$  peptides found in large amounts in fully developed Alzheimer cases, because of the low amounts of amyloid burden in the brain tissue of infracclinical cases (Delacourte et al. 2002). Aggregates consisting of amyloid-beta peptides (A $\beta$ ) in Alzheimer brains were soluble only in pure formic acid, as already described (Delacourte et al., 2002; Kalback et al., 2002). Such formic acid-soluble A $\beta$  species were resolved by two-dimensional gel (2D) electrophoresis and characterized using a panel of specific A $\beta$  antibodies, which revealed that both A $\beta$  x-40 and A $\beta$  x-42 species were present (Fig. 2; A $\beta$ -40 and A $\beta$ -42 panels). Further characterization was performed with brain tissue of Alzheimer's disease (AD) patients in which the total amount of formic acid soluble A $\beta$  enabled subsequent mass spectrometry. Ten A $\beta$  spots were resolved by 2D electrophoresis (Fig. 2; Coomassie Blue panel), nine of which were identified by mass spectrometry (Table 3). They all corresponded to monomers of A $\beta$ . The immunodetected dimeric species at 8kDa was present in too low amounts for mass spectrometric analysis. The full-length A $\beta$  peptides corresponded to spot 1 and spot 2 (Table 3). Spots 3-7 and 9-10 corresponded to amino-terminal truncated and post-translationally modified variants of A $\beta$  (Table 3). The major truncated variants consisted of A $\beta$  starting at amino-acid positions 2 to 5 and 8 to 10. The post-translational modifications characterized were pyroglutamylation at position 3 and methylation (Table 3). Interestingly, spots 6, 7, 9 and 10 contained similar A $\beta$  variants but were separated as two spots, suggesting that an as yet unidentified modification was present.

Coomassie Blue staining enabled a precise quantification of each A $\beta$  species. The full-length A $\beta$  peptides represented only 33% of all A $\beta$  species. The truncated variants thus accounted for more than 65%, among which 16% and 23% corresponded to truncated species starting at residues 4, 5, 9, and 10, respectively. Moreover, the 2D pattern of A $\beta$ <sub>40</sub> as revealed by the ADA40 antiserum completely overlapped the pattern obtained with WO2, which detects the amino-terminal region of A $\beta$ . These

results show that the identified truncated A $\beta$  derive from the A $\beta$ <sub>42</sub> and not A $\beta$ <sub>40</sub> species.

3. Characterization of A $\beta$  peptides in brain obtained from non-demented patients

Subsequently, the A $\beta$  species that aggregate in the first steps of amyloidosis were investigated in the brain tissue of non-demented patients. We studied fives cases that had traces to low amounts of A $\beta$ . A $\beta$  aggregates were exclusively comprised of A $\beta$ <sub>42</sub> species (Fig. 3; A $\beta$ <sub>40</sub> and A $\beta$ <sub>42</sub> panels), indicating a complete absence of A $\beta$ <sub>40</sub> species at infraclinical stages of Alzheimer pathology. Antibodies against the N-terminal region of A $\beta$  only detected a single spot corresponding to the full-length A $\beta$  peptide (Fig. 3; N-ter (5-8) and N-ter (4-13) panels). In addition, the A $\beta$ <sub>42</sub> specific antibody 21F12 labelled spots 4, 5, 6 and 10 (Fig. 3; A $\beta$ <sub>42</sub> panel) as well as dimers. The A $\beta$ <sub>42</sub> species in the brain of non-demented patients, as in Alzheimer's brain, correspond to N-terminal truncated variants starting at position 3-pyroglutamyl, 4, 5, 8 and 9. The lack of staining of A $\beta$  dimers with N-terminal A $\beta$  antibodies (Fig. 3 and Fig.4; N-ter (5-8) panel) demonstrates that A $\beta$  dimers are exclusively composed of N-terminally truncated A $\beta$ <sub>42</sub> species (Fig. 3 and Fig. 4; A $\beta$ <sub>42</sub> panel).

These A $\beta$ <sub>42</sub> variants do not result from treatment of brain tissue with formic acid (Delacourte et al., 2002; Kalback et al., 2002), as the treatment of synthetic A $\beta$  peptides 1-40 and 1-42 with formic acid did not generate the truncated variants derived from human brain tissue (Delacourte et al., 2002).

**Example 2: Generation of antibodies that specifically recognize N-terminal truncated and/or post-translationally modified peptides**

Peptide synthesis can be performed by Neosystems (Strasbourg, France). Following peptides are synthesized:

SGYEVHHQGC (A $\beta$  (8-15); SEQ ID NO 109);

HDSGYEVHGC (A $\beta$  (6-13); SEQ ID NO 83);

RHD SGY EVGC (A $\beta$  (5-12); SEQ ID NO 70).

These peptides are used as an immunogen to generate antibodies which specifically bind a N-terminal truncated and/post-translationally modified A $\beta$  variant of the invention. Rabbits are immunized with said peptides emulsified in Freund's adjuvant. The rabbits are bled and antibody titers are assayed by ELISA. The immunoglobulin fraction is isolated by standard ammoniumsulphate precipitation. The antibodies specific for one of the peptides can be purified by affinity chromatography.

In another experiment, immunization of mouse with the above peptides is undertaken. Isolation of splenic antibody producing cells is then carried out. These cells are fused to a cell characterized by immortality, such as a myeloma cell, to provide a fused cell hybrid (Hybridoma) which can be maintained in culture and which secretes the required monoclonal antibody. The cells are then cultured in bulk and the monoclonal antibodies harvested from the culture media for use.

Screening for the desired antibody is done by techniques known in the art such as ELISA. Antibodies are selected that bind with a 5 fold greater affinity to N-terminal truncated and/or post-translationally modified A $\beta$  variant of the invention compared to their binding to normal A $\beta$  peptide.

**TABLES**

**Table 1.** N-terminal truncated and/or post-translationally modified A $\beta$  peptides comprised in the preparation of the invention.

5

<b>Position in <math>\beta</math>A</b>	<b>Modification</b>	<b>Sequence</b>	<b>SEQ ID NO</b>
1-4	Methyl	DAEF	1
1-5	Methyl	DAEFR	2
1-6	Methyl	DAEFRH	3
1-7	Methyl	DAEFRHD	4
1-8	Methyl	DAEFRHDS	5
1-9	Methyl	DAEFRHDSG	6
1-10	Methyl	DAEFRHDSGY	7
1-11	Methyl	DAEFRHDSGYE	8
1-12	Methyl	DAEFRHDSGYEV	9
1-13	Methyl	DAEFRHDSGYEVH	10
1-14	Methyl	DAEFRHDSGYEVHH	11
1-15	Methyl	DAEFRHDSGYEVHHQ	12
1-16	Methyl	DAEFRHDSGYEVHHQK	13
2-5	None/methyl	AEFR	14
2-6	None/methyl	AEFRH	15
2-7	None/methyl	AEFRHD	16
2-8	None/methyl	AEFRHDS	17
2-9	None/methyl	AEFRHDSG	18
2-10	None/methyl	AEFRHDSGY	19
2-11	None/methyl	AEFRHDSGYE	20
2-12	None/methyl	AEFRHDSGYEV	21
2-13	None/methyl	AEFRHDSGYEVH	22
2-14	None/methyl	AEFRHDSGYEVHH	23
2-15	None/methyl	AEFRHDSGYEVHHQ	24
2-16	None/methyl	AEFRHDSGYEVHHQK	25
2-17	None/methyl	AEFRHDSGYEVHHQKL	26
3-6	None	EFRH	27
3-7	None	EFRHD	28
3-8	None	EFRHDS	29
3-9	None	EFRHDSG	30
3-10	None	EFRHDSGY	31
3-11	None	EFRHDSGYE	32
3-12	None	EFRHDSGYEV	33
3-13	None	EFRHDSGYEVH	34
3-14	None	EFRHDSGYEVHH	35
3-15	None	EFRHDSGYEVHHQ	36
3-16	None	EFRHDSGYEVHHQK	37
3-17	None	EFRHDSGYEVHHQKL	38
3-18	None	EFRHDSGYEVHHQKLV	39

**Table 1.** Continued

3-6	Pyroglutamyl	PyrE-EFRH	40
3-7	Pyroglutamyl	PyrE-EFRHD	41
3-8	Pyroglutamyl	PyrE-EFRHDS	42
3-9	Pyroglutamyl	PyrE-EFRHDSG	43
3-10	Pyroglutamyl	PyrE-EFRHDSGY	44
3-11	Pyroglutamyl	PyrE-EFRHDSGYE	45
3-12	Pyroglutamyl	PyrE-EFRHDSGYEV	46
3-13	Pyroglutamyl	PyrE-EFRHDSGYEVH	47
3-14	Pyroglutamyl	PyrE-EFRHDSGYEVHH	48
3-15	Pyroglutamyl	PyrE-EFRHDSGYEVHHQ	49
3-16	Pyroglutamyl	PyrE-EFRHDSGYEVHHQK	50
3-17	Pyroglutamyl	PyrE-EFRHDSGYEVHHQKL	51
3-18	Pyroglutamyl	PyrE-EFRHDSGYEVHHQKLV	52
4-7	None/methyl	FRHD	53
4-8	None/methyl	FRHDS	54
4-9	None/methyl	FRHDSG	55
4-10	None/methyl	FRHDSGY	56
4-11	None/methyl	FRHDSGYE	57
4-12	None/methyl	FRHDSGYEV	58
4-13	None/methyl	FRHDSGYEVH	59
4-14	None/methyl	FRHDSGYEVHH	60
4-15	None/methyl	FRHDSGYEVHHQ	61
4-16	None/methyl	FRHDSGYEVHHQK	62
4-17	None/methyl	FRHDSGYEVHHQKL	63
4-18	None/methyl	FRHDSGYEVHHQKLV	64
4-19	None/methyl	FRHDSGYEVHHQKLVF	65
5-8	None/methyl	RHDS	66
5-9	None/methyl	RHDSG	67
5-10	None/methyl	RHDSGY	68
5-11	None/methyl	RHDSGYE	69
5-12	None/methyl	RHDSGYEV	70
5-13	None/methyl	RHDSGYEVH	71
5-14	None/methyl	RHDSGYEVHH	72
5-15	None/methyl	RHDSGYEVHHQ	73
5-16	None/methyl	RHDSGYEVHHQK	74
5-17	None/methyl	RHDSGYEVHHQKL	75
5-18	None/methyl	RHDSGYEVHHQKLV	76
5-19	None/methyl	RHDSGYEVHHQKLVF	77
5-20	None/methyl	RHDSGYEVHHQKLVFF	78
6-9	None/methyl	HDSG	79
6-10	None/methyl	HDSGY	80
6-11	None/methyl	HDSGYE	81
6-12	None/methyl	HDSGYEV	82
6-13	None/methyl	HDSGYEVH	83
6-14	None/methyl	HDSGYEVHH	84
6-15	None/methyl	HDSGYEVHHQ	85

**Table 1.** Continued

6-16	None/methyl	HDSGYEVHHQK	86
6-17	None/methyl	HDSGYEVHHQKL	87
6-18	None/methyl	HDSGYEVHHQKLV	88
6-19	None/methyl	HDSGYEVHHQKLVF	89
6-20	None/methyl	HDSGYEVHHQKLVFF	90
6-21	None/methyl	HDSGYEVHHQKLVFFA	91
7-10	None/methyl	DSGY	92
7-11	None/methyl	DSGYE	93
7-12	None/methyl	DSGYEV	94
7-13	None/methyl	DSGYEVH	95
7-14	None/methyl	DSGYEVHH	96
7-15	None/methyl	DSGYEVHHQ	97
7-16	None/methyl	DSGYEVHHQK	98
7-17	None/methyl	DSGYEVHHQKL	99
7-18	None/methyl	DSGYEVHHQKLV	100
7-19	None/methyl	DSGYEVHHQKLVF	101
7-20	None/methyl	DSGYEVHHQKLVFF	102
7-21	None/methyl	DSGYEVHHQKLVFFA	103
7-22	None/methyl	DSGYEVHHQKLVFFAE	104
8-11	None	SGYE	105
8-12	None	SGYEV	106
8-13	None	SGYEVH	107
8-14	None	SGYEVHH	108
8-15	None	SGYEVHHQ	109
8-16	None	SGYEVHHQK	110
8-17	None	SGYEVHHQKL	111
8-18	None	SGYEVHHQKLV	112
8-19	None	SGYEVHHQKLVF	113
8-20	None	SGYEVHHQKLVFF	114
8-21	None	SGYEVHHQKLVFFA	115
8-22	None	SGYEVHHQKLVFFAE	116
8-23	None	SGYEVHHQKLVFFAED	117
9-12	None	GYEV	118
9-13	None	GYEVH	119
9-14	None	GYEVHH	120
9-15	None	GYEVHHQ	121
9-16	None	GYEVHHQK	122
9-17	None	GYEVHHQKL	123
9-18	None	GYEVHHQKLV	124
9-19	None	GYEVHHQKLVF	125
9-20	None	GYEVHHQKLVFF	126
9-21	None	GYEVHHQKLVFFA	127
9-22	None	GYEVHHQKLVFFAE	128
9-23	None	GYEVHHQKLVFFAED	129
9-24	None	GYEVHHQKLVFFAEDV	130
10-13	None	YEVH	131

**Table 1.** Continued

10-14	None	YEVHH	132
10-15	None	YEVHHQ	133
10-16	None	YEVHHQK	134
10-17	None	YEVHHQKL	135
10-18	None	YEVHHQKLV	136
10-19	None	YEVHHQKLVF	137
10-20	None	YEVHHQKLVFF	138
10-21	None	YEVHHQKLVFFA	139
10-22	None	YEVHHQKLVFFAE	140
10-23	None	YEVHHQKLVFFAED	141
10-24	None	YEVHHQKLVFFAEDV	142
10-25	None	YEVHHQKLVFFAEDVG	143
1-42	Methyl	DAEFRHDSGYEVHHQLVFFAEDVGSNKGAIIIGLMVGGVVIA	144
2-42	None/methyl	AEFRHDSGYEVHHQLVFFAEDVGSNKGAIIIGLMVGGVVIA	145
3-42		EFRHDSGYEVHHQLVFFAEDVGSNKGAIIIGLMVGGVVIA	146
3-42	Pyroglutamyl	PyrE-EFRHDSGYEVHHQLVFFAEDVGSNKGAIIIGLMVGGVVIA	147
4-42	None/methyl	FRHDSGYEVHHQLVFFAEDVGSNKGAIIIGLMVGGVVIA	148
5-42	None/methyl	RHDSGYEVHHQLVFFAEDVGSNKGAIIIGLMVGGVVIA	149
6-42	None/methyl	HDSGYEVHHQLVFFAEDVGSNKGAIIIGLMVGGVVIA	150
7-42	None/methyl	DSGYEVHHQLVFFAEDVGSNKGAIIIGLMVGGVVIA	151
8-42		SGYEVHHQLVFFAEDVGSNKGAIIIGLMVGGVVIA	152
9-42		GYEVHHQLVFFAEDVGSNKGAIIIGLMVGGVVIA	153
10-42		YEVHHQLVFFAEDVGSNKGAIIIGLMVGGVVIA	154
1-40	Methyl	DAEFRHDSGYEVHHQLVFFAEDVGSNKGAIIIGLMVGGVVIA	155
2-40	None/methyl	AEFRHDSGYEVHHQLVFFAEDVGSNKGAIIIGLMVGGVVIA	156
3-40		EFRHDSGYEVHHQLVFFAEDVGSNKGAIIIGLMVGGVVIA	157
3-40	Pyroglutamyl	PyrE-EFRHDSGYEVHHQLVFFAEDVGSNKGAIIIGLMVGGVVIA	158
4-40	None/methyl	FRHDSGYEVHHQLVFFAEDVGSNKGAIIIGLMVGGVVIA	159
5-40	None/methyl	RHDSGYEVHHQLVFFAEDVGSNKGAIIIGLMVGGVVIA	160

**Table 1.** Continued

6-40	None/methyl	HDSGYEVHHQLVFFAEDVGSNKGAIIGL MVGGVV	161
7-40	None/methyl	DSGYEVHHQLVFFAEDVGSNKGAIIGLM VGGVV	162
8-40		SGYEVHHQLVFFAEDVGSNKGAIIGLMV GGVV	163
9-40		GYEVHHQLVFFAEDVGSNKGAIIGLMVG GVV	164
10-40		YEVHHQLVFFAEDVGSNKGAIIGLMVGG VV	165

**Table 2.** C-terminal fragments of the N-terminal APP soluble fragments of the invention.

Positions: APP - $\beta$ -amyloid	Sequence	SEQ ID NO
669-1	VKMD	166
668-1	EVKMD	167
667-1	SEVKMD	168
666-1	ISEVKMD	169
656-1	EISEVKMD	170
664-1	EEISEVKMD	171
663-1	TEEISEVKMD	172
662-1	KTEEISEVKMD	173
661-1	IKTEEISEVKMD	174
660-1	NIKTEEISEVKMD	175
659-1	TNIKTEEISEVKMD	176
658-1	LTKNIKTEEISEVKMD	177
657-1	GLTNIKTEEISEVKMD	178
670-2	KMDA	179
669-2	VKMDA	180
668-2	EVKMDA	181
667-2	SEVKMDA	182
666-2	ISEVKMDA	183
665-2	EISEVKMDA	184
664-2	EEISEVKMDA	185
663-2	TEEISEVKMDA	186
662-2	KTEEISEVKMDA	187
661-2	IKTEEISEVKMDA	188
660-2	NIKTEEISEVKMDA	189
659-2	TNIKTEEISEVKMDA	190
658-2	LTKNIKTEEISEVKMDA	191
671-3	MDAE	192
670-3	KMDAE	193
669-3	VKMDAE	194
668-3	EVKMDAE	195
667-3	SEVKMDAE	196
666-3	ISEVKMDAE	197
665-3	EISEVKMDAE	198
664-3	EEISEVKMDAE	199
663-3	TEEISEVKMDAE	200
662-3	KTEEISEVKMDAE	201
661-3	IKTEEISEVKMDAE	202
660-3	NIKTEEISEVKMDAE	203
659-3	TNIKTEEISEVKMDAE	204
672-4	DAEF	1
671-4	MDAEF	205
670-4	KMDAEF	206

**Table 2.** Continued

669-4	VKMDAEC	207
668-4	EVKMDAEC	208
667-4	SEVKMDAEC	209
666-4	ISEVKMDAEC	210
665-4	EISEVKMDAEC	211
664-4	EEISEVKMDAEC	212
663-4	TEEISEVKMDAEC	213
662-4	KTEEISEVKMDAEC	214
661-4	IKTEEISEVKMDAEC	215
660-4	NIKTEEISEVKMDAEC	216
673-5	AEFR	14
672-5	DAEFR	2
671-5	MDAEFR	217
670-5	KMDAEFR	218
669-5	VKMDAEFR	219
668-5	EVKMDAEFR	220
667-5	SEVKMDAEFR	221
666-5	ISEVKMDAEFR	222
665-5	EISEVKMDAEFR	223
664-5	EEISEVKMDAEFR	224
663-5	TEEISEVKMDAEFR	225
662-5	KTEEISEVKMDAEFR	226
661-5	IKTEEISEVKMDAEFR	227
674-6	EFRH	27
673-6	AEFRH	15
672-6	DAEFRH	3
671-6	MDAEFRH	228
670-6	KMDAEFRH	229
669-6	VKMDAEFRH	230
668-6	EVKMDAEFRH	231
667-6	SEVKMDAEFRH	232
666-6	ISEVKMDAEFRH	233
665-6	EISEVKMDAEFRH	234
664-6	EEISEVKMDAEFRH	235
663-6	TEEISEVKMDAEFRH	236
662-6	KTEEISEVKMDAEFRH	237
675-7	FRHD	53
674-7	EFRHD	28
673-7	AEFRHD	16
672-7	DAEFRHD	4
671-7	MDAEFRHD	238
670-7	KMDAEFRHD	239
669-7	VKMDAEFRHD	240
668-7	EVKMDAEFRHD	241
667-7	SEVKMDAEFRHD	242
666-7	ISEVKMDAEFRHD	243

**Table 2.** Continued

665-7	EISEVKMDAEFRHD	244
664-7	EEISEVKMDAEFRHD	245
663-7	TEEISEVKMDAEFRHD	246
676-8	RHDS	66
675-8	FRHDS	54
674-8	EFRHDS	29
673-8	AEFRHDS	17
672-8	DAEFRHDS	5
671-8	MDAEFRHDS	247
670-8	KMDAEFRHDS	248
669-8	VKMDAEFRHDS	249
668-8	EVKMDAEFRHDS	250
667-8	SEVKMDAEFRHDS	251
666-8	ISEVKMDAEFRHDS	252
665-8	EISEVKMDAEFRHDS	253
664-8	EEISEVKMDAEFRHDS	254
677-9	HDSG	79
676-9	RHDSG	67
675-9	FRHDSG	55
674-9	EFRHDSG	30
673-9	AEFRHDSG	18
672-9	DAEFRHDSG	6
671-9	MDAEFRHDSG	255
670-9	KMDAEFRHDSG	256
669-9	VKMDAEFRHDSG	257
668-9	EVKMDAEFRHDSG	258
667-9	SEVKMDAEFRHDSG	259
666-9	ISEVKMDAEFRHDSG	260
665-9	EISEVKMDAEFRHDSG	261

**Table 3.** Digested peptides of A $\beta$  variants present in Alzheimer's disease patients

Spot	A $\beta$ proposed identity <sup>1</sup>	Theoretical mass	Observed mass	Relative amount <sup>2</sup>	Theoretical pI	Observed pI
1	1-16	1954.879	1954.875	23%	5.31	5.3
	1-16+CH <sub>3</sub>	1968.905	1968.863	-	5.31	5.3
2	1-16	1954.879	1954.875	10%	5.31	5.3
	1-16+CH <sub>3</sub>	1968.905	1968.863	-	5.31	5.3
3	2-16	1839.852	1839.851	6%	5.78	5.8
	2-16+CH <sub>3</sub>	1853.878	1853.854	-	5.78	5.8
	(3-16)	1768.815	1768.804	-	5.78	5.8
4	pyrE 3-16	1751.784	1750.790	10%	6.27	5.9
	(2-16)	1839.852	1839.833	-	5.78	5.9
5	pyrE 3-16	1751.784	1750.881	8%	6.27	6.3
6	8-16	1084.517	1084.557	10%	5.96	6.0
	9-16	997.485	998.525	-	6.01	6.0
7	8-16	1084.517	1084.518	13%	5.96	6.1
	9-16	997.485	997.477	-	6.01	6.1
	10-16	940.463	940.460	-	6.01	6.1
9&10	4-16	1639.772	1639.848	16%	6.27	6.3
	4-16+CH <sub>3</sub>	1653.798	1653.859	-	6.27	6.3
	5-16	1492.704	1492.770	-		

<sup>1</sup>Methylated fragments are indicated with a CH<sub>3</sub>. PyrE corresponds to a pyroglutamyl residue at the N-terminus of the identified fragment. The peptide corresponding to

5 amino acid sequence 17-28 of A $\beta$  was found in all spots (not shown).

<sup>2</sup>The relative amount corresponds to the quantification using Melanie III software on Coomassie stained gels.

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## CLAIMS

1. A preparation comprising a  $\beta$ -amyloid variant or a N-terminal fragment thereof characterized in that said  $\beta$ -amyloid variant or said N-terminal fragment thereof 5 contains a truncation and/or a post-translational modification at its N-terminus.
2. The preparation according to claim 1, further characterized in that the N-terminal truncated  $\beta$ -amyloid variant or the N-terminal fragment thereof starts at position 2, 10 3, 4, 5, 6, 7, 8, 9 or 10 of  $\beta$ -amyloid and that the post-translational modification is a methylation or a pyroglutamylolation.
3. The preparation according to claim 2, further characterized in that the 15 pyroglutamylation is present at position 3 of a N-terminal truncated  $\beta$ -amyloid variant starting at position 3 of  $\beta$ -amyloid.
4. The preparation according to any of claims 1 to 3, further characterized in that the 20  $\beta$ -amyloid variant or the N-terminal fragment thereof comprises an amino acid sequence selected from the group consisting of SEQ ID NOs 1 to 165.
5. The preparation according to any of claims 1 to 4, further characterized in that the 25  $\beta$ -amyloid variant or the N-terminal fragment thereof contains an additional modification resulting in a separated spot of the  $\beta$ -amyloid variant or the N-terminal fragment thereof on two-dimensional gel electrophoresis compared to the spot obtained with the  $\beta$ -amyloid variant or the N-terminal fragment thereof without said additional modification.
6. A preparation comprising a N-terminal APP soluble fragment obtainable by secretase cleavage of APP, characterized in that the C-terminal end of said N-terminal APP soluble fragment consists of position 1, 1 to 2, 1 to 3, 1 to 4, 1 to 5, 30 1 to 6, 1 to 7, 1 to 8 or 1 to 9 of  $\beta$ -amyloid, a preparation comprising a C-terminal fragment thereof.

7. The preparation according to claims 6, further characterized in that the N-terminal APP soluble fragment or the C-terminal fragment thereof comprises an amino acid sequence selected from the group consisting of SEQ ID NOs 1 to 6, 14 to 18, 27 to 30, 53 to 55, 66 to 67, 79, and 166 to 261.

5

8. A nucleic acid preparation comprising a nucleic acid sequence capable of encoding the  $\beta$ -amyloid variant or the N-terminal fragment thereof according to any of claims 1 to 5.

10 9. A nucleic acid preparation comprising a nucleic acid sequence capable of encoding the N-terminal APP soluble fragment or the C-terminal fragment thereof according to any of claims 6 or 7.

10. A method for the preparation of an antibody that specifically recognizes a N-terminally truncated and/or post-translationally modified  $\beta$ -amyloid variant, comprising the following steps:

(a) Immunizing an animal with a preparation according to any of claims 1 to 5 or with the nucleic acid preparation according to claim 8;

(b) Obtaining the antibodies generated by the immunization in step (a);

20 (c) Screening the antibodies obtained in step (b) for their specific recognition of N-terminal truncated and/or post-translationally modified  $\beta$ -amyloid variants.

11. An antibody obtainable by the method according to claim 10.

25 12. A method for the preparation of an antibody that specifically recognizes a N-terminal APP soluble fragment according to any of claims 6 or 7, comprising the following steps:

(a) Immunizing an animal with a preparation of N-terminal APP soluble fragment or a C-terminal fragment thereof according to any of claims 6 or 7 or with the nucleic acid preparation according to claim 9;

(b) Obtaining the antibodies generated by the immunization in step (a);

30 (c) Screening the antibodies obtained in step (b) for their specific recognition of N-terminal APP soluble fragment according to any of claims 6 or 7.

13. An antibody obtainable by the method according to claim 12.

14. A vaccine composition or a therapeutic composition comprising a preparation  
5 according to any of claims 1 to 5, comprising an antibody according to claim 11,  
or comprising a nucleic acid preparation according to claim 8.

15. A preparation according to any of claims 1 to 5, an antibody according to claim  
10 11, or a nucleic acid preparation according to claim 8 for use as a prophylactic  
vaccine for the prevention of a disease associated with  $\beta$ -amyloid formation  
and/or aggregation such as Alzheimer's disease. [REDACTED]

16. A preparation according to any of claims 1 to 5, an antibody according to claim  
11, or a nucleic acid preparation according to claim 8 for use as a therapeutic for  
15 the treatment of a disease associated with  $\beta$ -amyloid formation and/or aggregation  
such as Alzheimer's disease.

17. Use of a preparation according to any of claims 1 to 5, an antibody according to  
claim 11, or a nucleic acid preparation according to claim 8 for the manufacture of  
20 a prophylactic vaccine for the prevention of a disease associated with  $\beta$ -amyloid  
formation and/or aggregation such as Alzheimer's disease.

18. Use of a preparation according to any of claims 1 to 5, an antibody according to  
claim 11, or a nucleic acid preparation according to claim 8 for the manufacture of  
25 a therapeutic for the treatment of a disease associated with  $\beta$ -amyloid formation  
and/or aggregation such as Alzheimer's disease.

19. A method for the prevention and/or treatment, in an mammal, of a disease  
associated with  $\beta$ -amyloid formation and/or aggregation such as Alzheimer's  
30 disease, said method comprising the administration, to said mammal, of a vaccine  
composition or a therapeutic composition according to claim 14.

20. A diagnostic or theranostic kit comprising a preparation according to any of claims 1 to 5, comprising a preparation according to any of claim 6 or 7, or comprising an antibody according to any of claims 11 or 13.
- 5     21. A preparation according to any of claims 1 to 5 for use as a diagnostic or theranostic for the measurement of the immune response induced in a mammal by vaccination or therapeutic application with respectively a vaccine composition or a therapeutic composition according to claim 14.
- 10    22. Use of a preparation according to any of claims 1 to 5 for the manufacture of a diagnostic or theranostic kit for the measurement of the immune response induced in a mammal by vaccination or therapeutic application with respectively a vaccine composition or a therapeutic composition according to claim 14.
- 15    23. A method for the measurement, in a mammal, of the immune response induced by vaccination or therapeutic application with respectively a vaccine composition or a therapeutic composition according to claim 14, said method comprising the following steps:
- 20       (a) Determining, in a sample obtained from said mammal, the amount of antibody specific for a  $\beta$ -amyloid variant according to any of claims 1 to 5;
- 25       (b) Comparing the amount determined in step (a) with the amount of antibody specific for said  $\beta$ -amyloid variant present in the mammal before vaccination or therapeutic application with the vaccine or therapeutic composition according to claim 14;
- 30       (c) Concluding, from the comparison in step (b), whether the mammal is responding to the vaccination or therapy, an increased amount of antibody specific for said  $\beta$ -amyloid variant being an indication that the mammal is responding to the vaccination or therapy.
- 30    24. A preparation according to any of claims 1 to 5, an antibody according to claim 11 or 13, or a preparation according to any of claims 6 or 7 for use as a diagnostic or theranostic for determining, in a mammal, the susceptibility to a disease associated with  $\beta$ -amyloid formation and/or aggregation such as Alzheimer's disease, for

determining, in a mammal, the risk of developing a disease associated with  $\beta$ -amyloid formation and/or aggregation such as Alzheimer's disease, for screening of the clearance of  $\beta$ -amyloid deposition in a mammal, or for predicting the level of  $\beta$ -amyloid burden in a mammal.

5

25. Use of a preparation according to any of claims 1 to 5, an antibody according to claim 11 or 13, or a preparation according to any of claims 6 or 7 for the manufacture of diagnostic or theranostic kit for determining, in a mammal, the susceptibility to a disease associated with  $\beta$ -amyloid formation and/or aggregation such as Alzheimer's disease, for determining, in a mammal, the risk of developing

10 a disease associated with  $\beta$ -amyloid formation and/or aggregation such as Alzheimer's disease, for screening of the clearance of  $\beta$ -amyloid deposition in a mammal, or for predicting the level of  $\beta$ -amyloid burden in a mammal.

15 26. A method for determining, in a mammal, the susceptibility to a disease associated with  $\beta$ -amyloid formation and/or aggregation such as Alzheimer's disease, for determining, in a mammal, the risk of developing a disease associated with  $\beta$ -amyloid formation and/or aggregation such as Alzheimer's disease, for screening of the clearance of  $\beta$ -amyloid deposition in a mammal, or for predicting the level 20 of  $\beta$ -amyloid burden in a mammal, said method comprising the following steps:

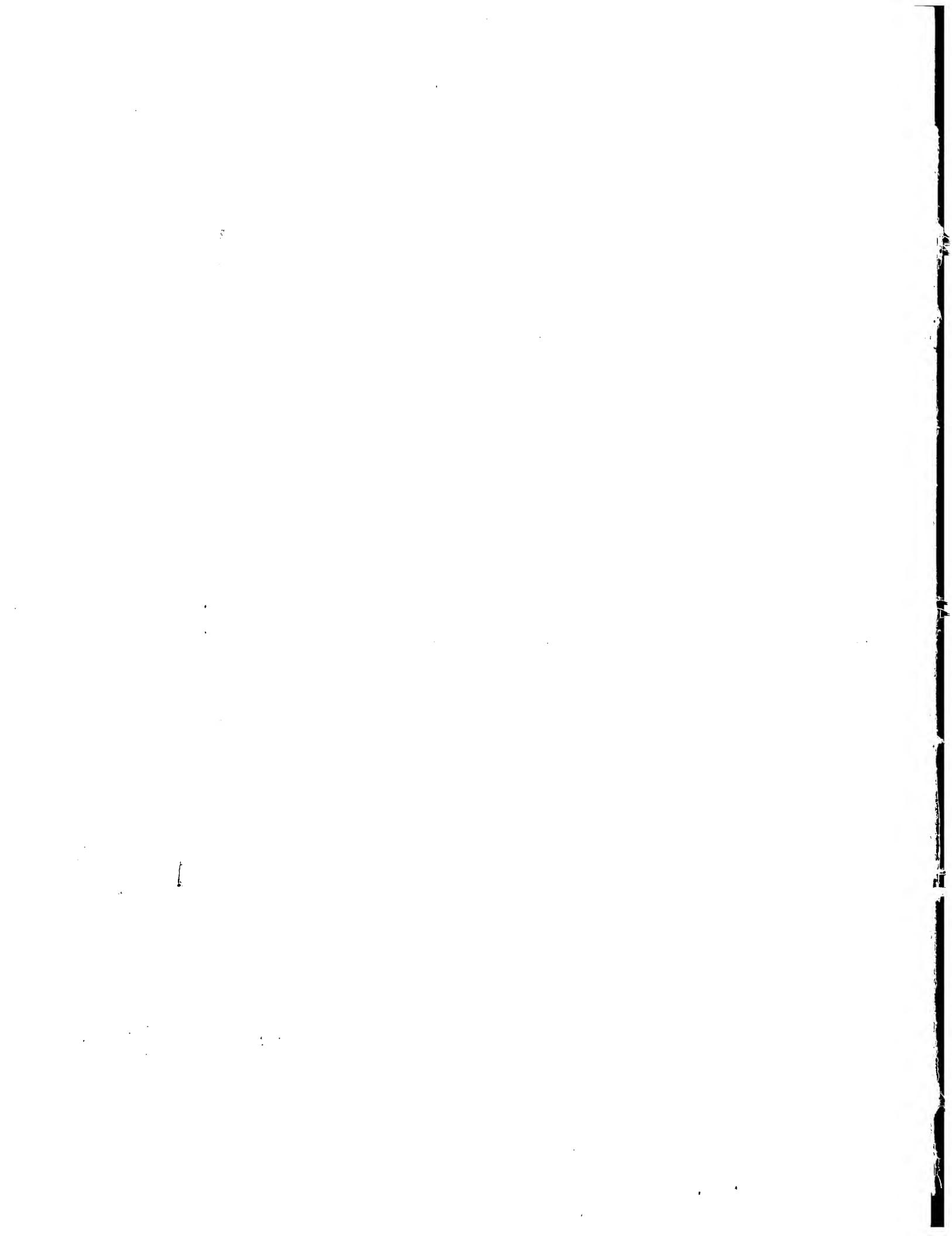
- (a) Determining, in said mammal, the amount of  $\beta$ -amyloid variant according to any of claims 1 to 5, the amount of N-terminal APP soluble fragment according to any of claims 6 or 7 or the amount of antibody specific for said  $\beta$ -amyloid variant or said APP soluble fragment;
- (b) Comparing the amount determined in step (a) with the amount of said  $\beta$ -amyloid variant, said N-terminal APP soluble fragment, or said antibody in a control mammal;
- (c) Concluding, from the comparison in step (b), whether the mammal is susceptible to a disease associated with  $\beta$ -amyloid formation and/or aggregation such as Alzheimer's disease, whether the mammal is at risk of 30 developing a disease associated with  $\beta$ -amyloid formation and/or aggregation

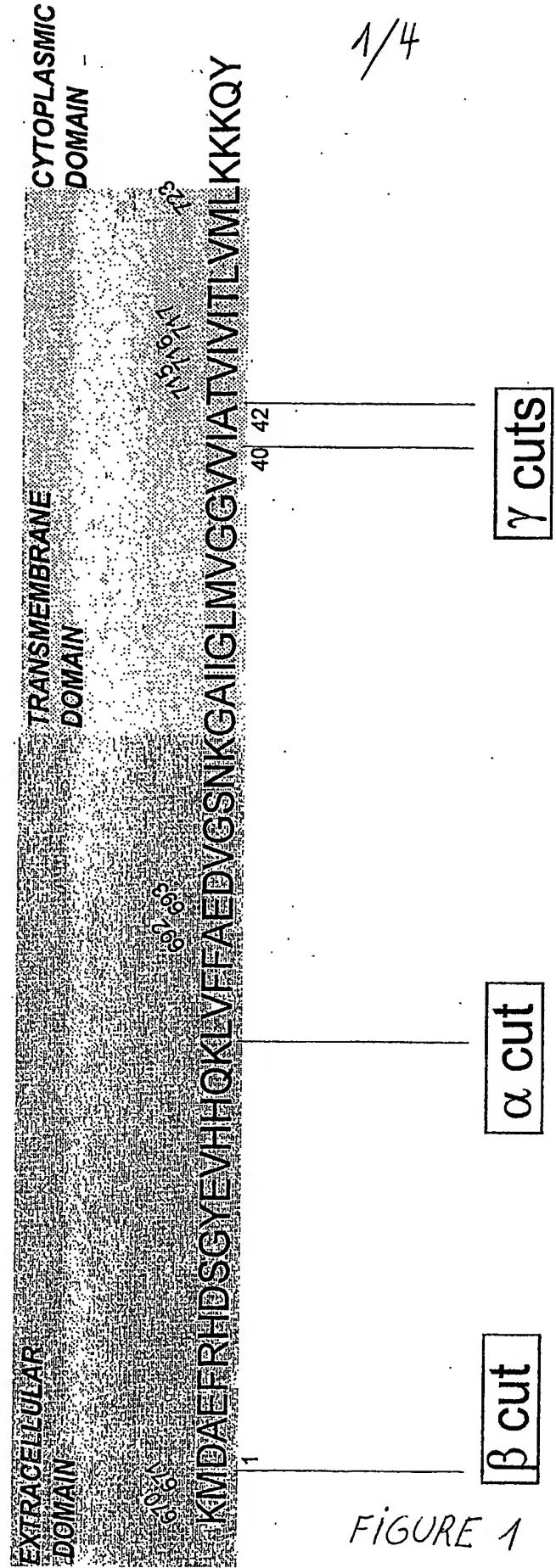
such as Alzheimer's disease, whether the  $\beta$ -amyloid deposition in the mammal is cleared, or what the level of  $\beta$ -amyloid burden is in said mammal.

27. The method according to claim 26, further characterized in that the amount of  $\beta$ -amyloid variant, the amount of N-terminal APP soluble fragment or the amount of antibody specific for said  $\beta$ -amyloid variant or said N-terminal APP soluble fragment is determined on a tissue sample obtained from said mammal.
- 10 28. The method according to claim 27 for predicting the level of  $\beta$ -amyloid burden in a mammal, said method comprising the following steps:
- (a) Administration, to said mammal, of a vaccine composition or a therapeutic composition according to claim 14;
  - (b) Determining, in a biological fluid sample obtained from said mammal, the amount of  $\beta$ -amyloid variant according to any of claims 1 to 5;
  - 15 (c) Comparing the amount determined in step (a) with the amount of said  $\beta$ -amyloid variant in a biological fluid sample obtained from a control mammal;
  - (d) Concluding, from the comparison in step (b), what the level of  $\beta$ -amyloid burden is in said mammal.

## **ABSTRACT**

The invention provides compositions and methods for prevention and treatment of diseases associated with  $\beta$ -amyloid formation and/or aggregation. Such methods 5 encompass the induction of an immune response against N-terminal truncated and/or post-translationally modified A $\beta$  peptides. These peptides are further used in compositions and methods for the diagnosis of diseases associated with  $\beta$ -amyloid formation and/or aggregation.





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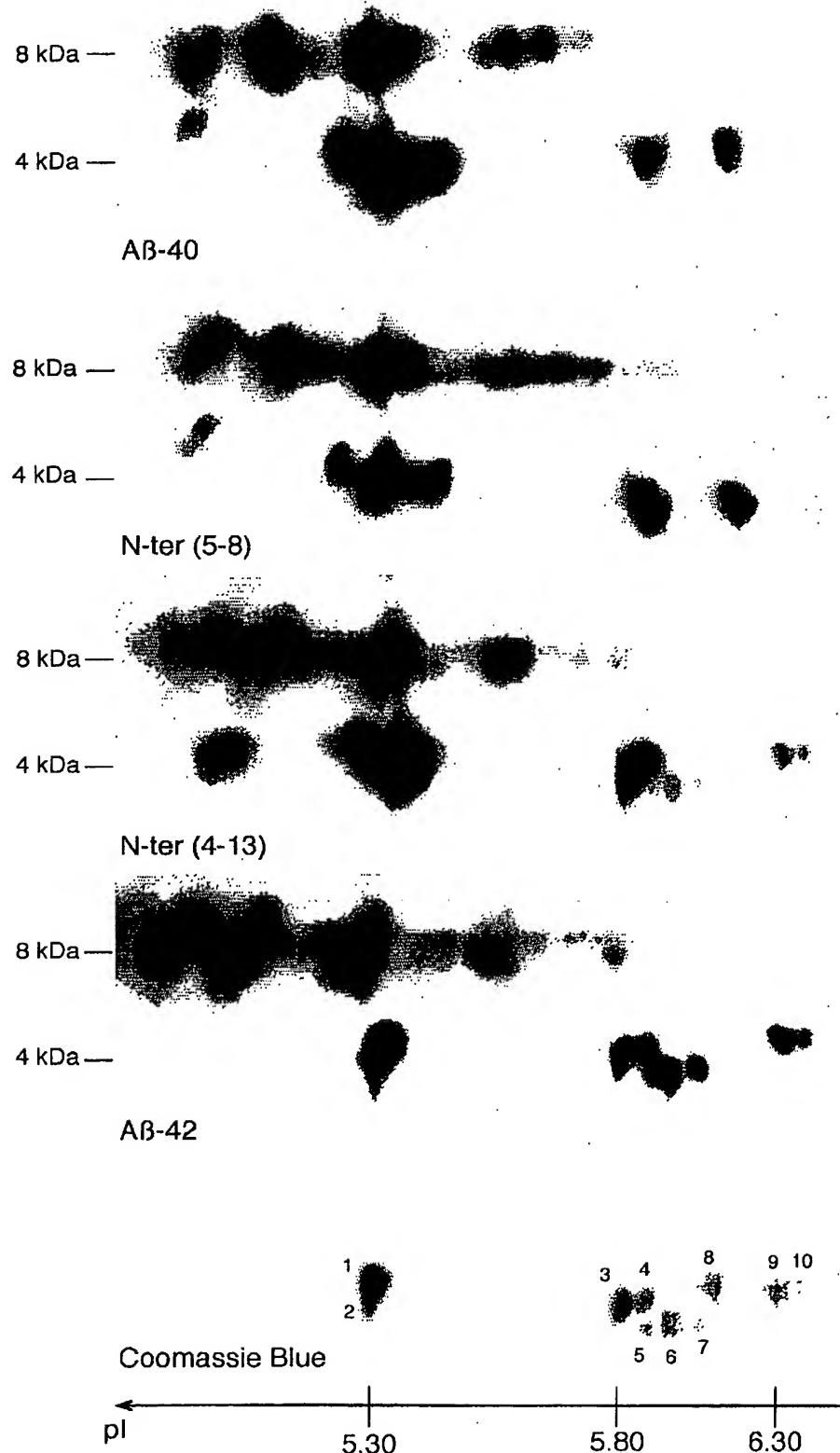
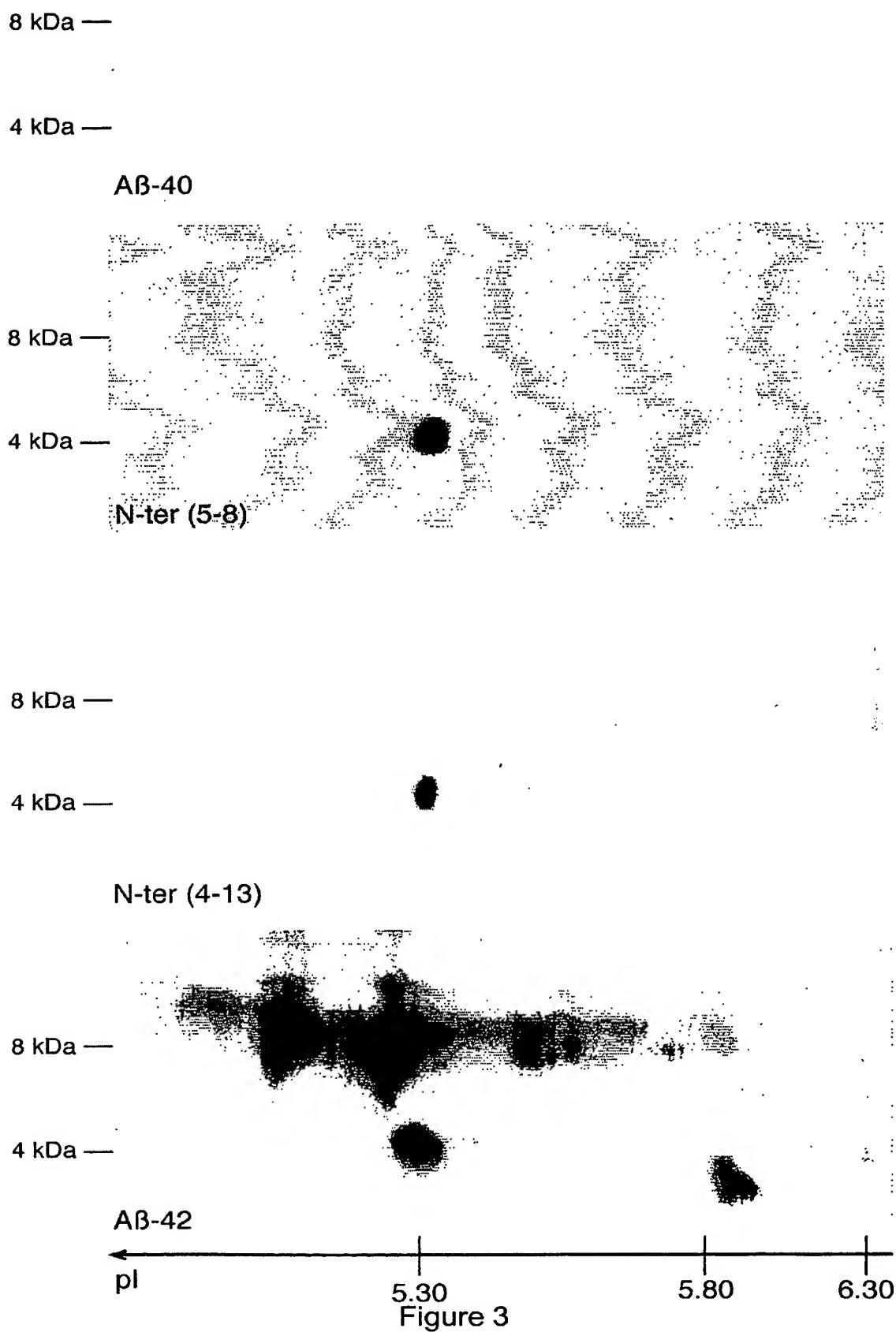


Figure 2



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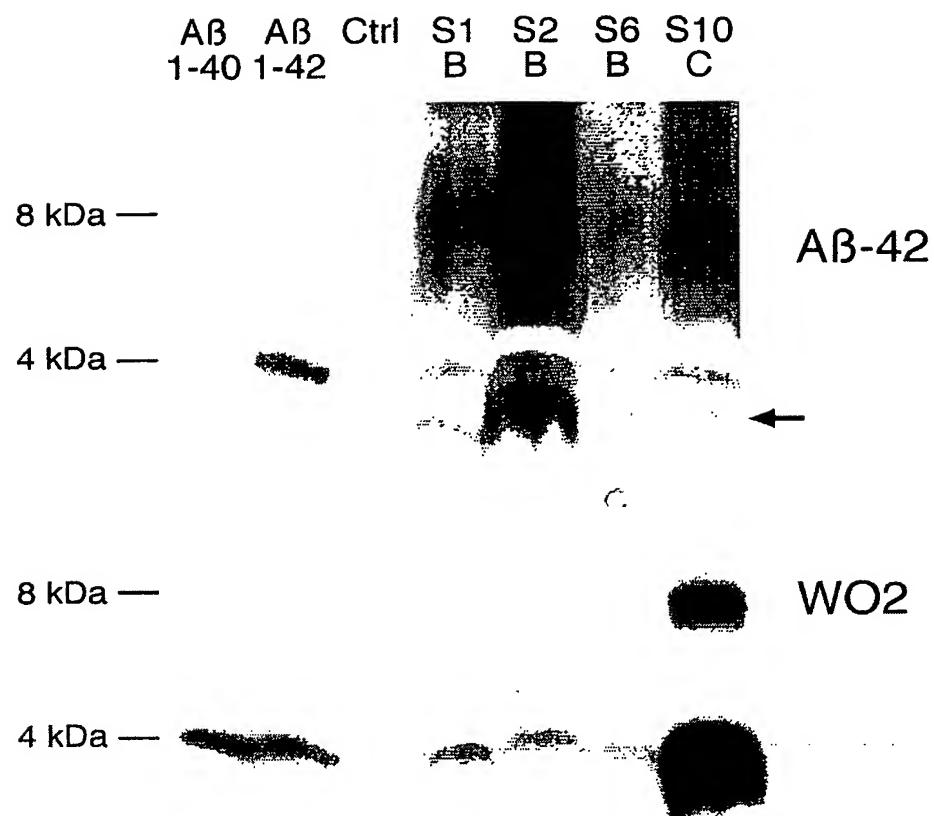


Figure 4